

The role of calcium and calcium antagonists in the reperfusion injury of the heart.

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Declaration.

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously, in its entirety or in part, submitted it at any university for a degree.

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Summary.

The reperfusion injury after myocardial ischemia is relevant in the clinical setting, after cardiopulmonary bypass for cardiac surgery, after PTCA and stenting and after cardiopulmonary resuscitation. The components of the reperfusion injury considered in this study were myocardial stunning and reperfusion arrhythmias.

Calcium antagonists have been shown to be beneficial in attenuating the myocardial reperfusion injury in the in vitro and in vivo laboratory setting (Lamping, Gross 1985, Przyklenk and Kloner 1988, Taylor 1990, Ehring 1992, Gross and Piper 1992). However systemic administration of a dose of calcium antagonist, large enough to attenuate the myocardial reperfusion injury in the clinical setting, would inevitably lead to unwanted systemic side effects of the drug.

The aim of this study was to investigate the hypothesis that an adequate dose of verapamil administered timeously, directly into the ischemic myocardium, would attenuate the reperfusion injury, either when administered from the onset of ischemia, or from 3 minutes before reperfusion.

The anesthetized open chest porcine model of myocardial ischemia (15 min total LAD occlusion) and reperfusion was employed in this study. A low dose of verapamil (0.5 mg/8ml or 0.0625mg/ml), a high dose of verapamil (2mg/8ml or

0.25mg/ml), or vehicle (saline) (8ml) was infused over 8 minutes, directly into the LAD coronary artery supplying the ischemic segment. The infusion was started either at the onset of ischemia, or from 3 minutes before reperfusion. The time taken for the various parameters to return to pre ischemic values was compared between the different groups.

The results showed that the high dose of verapamil (2mg) attenuated the reperfusion injury both when administered from the onset of ischemia, and when administered from 3 minutes before reperfusion, compared to either the low dose of verapamil, or the saline infusions. The high dose of verapamil groups had a faster recovery of both systolic contractile function and diastolic function and a lower incidence of ventricular fibrillation on reperfusion. There were no systemic effects of verapamil infusion in any of the groups.

The clinical setting of cardiac surgery expressly lends itself to the clinical application of this finding. There is direct access to the coronary arteries both before ischemia and before reperfusion. A small dose of calcium channel blocking drug, with no systemic effect can be administered into the aortic root at the onset of ischemia, just prior to cardioplegia (when the heart is still warm), and after rewarming a few minutes prior to removal of the aortic cross clamp.

Opsomming.

Die reperfusie besering na miokardiale isgemie is klinies relevant na kardiopulmonêre omleiding vir hart chirurgie, na kardiologiese PTKA en stut prosedures en na kardiopulmonale ressussitasie. Die komponente van die reperfusie besering wat in hierdie studie oorweeg is, is miokardiale tydelike omkeerbare onderdrukking (stunning) en reperfusie arritmieë.

Kalsium antagoniste is gewys om effektief te wees in beperking van die reperfusie besering in beide in vitro en in vivo laboratorium eksperimente (Lamping, Gross 1985, Przyklenk en Kloner 1988, Taylor 1990, Ehring 1992, Gross en Piper 1992). Sistemiese toediening van 'n dosis kalsium kanaal blokker, voldoende om die miokardiale reperfusie besering in die pasiënt te beperk, lei egter tot ongewenste sistemiese newe effekte van die middel.

Die doel van die studie was om die hipotese te ondersoek dat 'n voldoende dosis verapamil, wat betyds direk toegedien is aan die isgemiese miokardium, die reperfusie besering sal beperk, ongeag of dit toegedien is vanaf die begin van isgemie, of van 3 minute voor reperfusie.

Die vark model van miokardiale isgemie en reperfusie is aangewend in die studie. Die varke was tydens die eksperiment onder narkose, met die borskas oop, en 15 minute totale LAD okklusie is toegepas. 'n Lae dosis verapamil (0.5mg/8ml

of 0.0625 mg/ml), of hoë dosis verapamil (2mg/8ml of 0.25mg/ml), of saline (8ml) is oor 8 minute toegedien direk in die LAD arterie wat die isgemiese segment voorsien. Die infuus is begin direk na die aanvang van isgemie, of 3 minute voor die aanvang van reperfusie. Die tyd geneem vir terugvoer van parameters na pre isgemiese waardes is tussen die groepe vergelyk.

Die resultate toon dat die hoë dosis verapamil die reperfusie besering beperk in vergelyking met die lae dosis verapamil of saline infusies, ongeag of dit van die begin van isgemie, of van 3 minute voor reperfusie toegedien word. Die groepe wat die hoë dosis verapamil ontvang het, het vinniger herstel van sistoliese en diastoliese funksie getoon en het 'n laer insidensie van reperfusie disritmieë, gewys. Geen sistemiese effekte van verapamil infuus is waargeneem nie.

Die kliniese toepassing van hierdie bevinding is by uitstek geskik vir toepassing tydens kardiopulmonale omleiding by kardiaal chirurgie. Daar is direkte toegang tot koronêre arteries voor isgemie en voor reperfusie. 'n Klein dosis kalsium antagonist, met weglaatbare sistemiese effekte, kan toegedien word in die aorta wortel met die aanvang van isgemie, net voor kardioplegie toediening (hart steeds warm), en na verwarming, 'n paar minute voor verwydering van die aorta kruis klem.

1. Literature review

1.1 Calcium and the reperfusion injury.

1.2 Physiology.

1.3 Calcium channel blocking drugs and the reperfusion injury.

1.4 Hypothesis and proposed clinical application.

1.1 Calcium and reperfusion injury.

1.1.1 Introduction.

1.1.2 Pathophysiology of the reperfusion injury.

1.1.2.1 Ischemia.

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1.1.3.1 Mitochondrial permeability transition pore (MPTP) opening.

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1.1.3.4 Decreased nitric oxide (NO) production.

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1.1 Calcium and the reperfusion injury.

1.1.1 Introduction.

Experiments at Harvard Medical School in 1975 (Heyndrickx et al. 1975) in which the consequences of ischemia and reperfusion on myocardial function were examined, revealed prolonged dysfunction with eventual return of normal contractile activity once ischemia was relieved. In 1982 Braunwald and Kloner termed this condition *myocardial stunning*.

During the 1980's there was a growth of interest in this phenomenon due to the advent of thrombolytic therapy and other forms of interventional revascularization.

Early animal studies demonstrated that once the myocardium becomes severely ischemic, restoration of blood flow is a pre-requisite for myocardial salvage. However restoration of blood flow after transient ischemia is associated with arrhythmias, myocardial stunning (reversible prolonged dysfunction), acceleration of necrosis in cells, and microvascular injury. These events were termed reperfusion injury.

Despite the evidence of a detrimental reperfusion injury in animal studies, the clinical experience demonstrated that reperfusion therapy in patients with evolving acute myocardial infarction is always preferable to permanent coronary

occlusion. Also studies aimed at attenuating the effects of the reperfusion injury in the clinical setting yielded controversial results (Bolli, 1991, Rahimtoola 1985; Serruys 1984; Labovitz 1987). Therefore the reperfusion injury was considered by many to be either non-existent or clinically irrelevant compared to the ischemic injury.

However in contrast to cardiologists, many cardiovascular surgeons were convinced of the clinical relevance of the reperfusion injury in the clinical setting following cardioplegic arrest during surgery (García Dorado 2004).

The reperfusion injury must be differentiated from ischemic injury. Firm evidence for reperfusion injury would require:

- The appearance of an entirely new phenomenon at the time of reperfusion.
- Proof that a reperfusion associated event can be diminished in severity by an intervention given only at the time of reperfusion (Opie 1989).

In recent years, due to better understanding of the mechanisms of cell injury/death during myocardial ischemia/reperfusion, the reperfusion injury, as a well documented and clinically relevant phenomenon, is widely accepted.

The following are salient facts with regard to cardiomyocyte death associated with reperfusion (García Dorado 2004):

1. It occurs mainly during the initial minutes of reflow.
2. It involves rupture of the cell membrane.
3. It is due to mechanisms originating in the cardiomyocyte (not blood born cells).
4. It can be prevented by interventions applied at the time of reperfusion.

The reperfusion injury is relevant in the following clinical settings:

1. After acute coronary occlusion (normothermic).
2. After extra corporeal circulation (hypothermic).
3. After donor graft preservation (deep hypothermia).
4. During resuscitation after cardiac arrest.

1.1.2 Pathophysiology of the reperfusion injury.

Cellular mechanisms of the reperfusion injury:

1.1.2.1 Ischemia.

This is characterized by a rapid reduction in ATP content. When the cytosolic ATP decreases into the window between 100 μ M and zero, contracture of the myocytes develops. Shortly thereafter when the ATP stores are depleted, this

shortening stays fixed as the cross bridges between actin and myosin remain intact (Altschuld 1985, Nichols 1990). This ischemic contracture is moderate and causes no structural damage. It does however make the myocyte more fragile and therefore susceptible to mechanical damage (Schlüter 1996) (Fig. 1.3).

Consequences of ischemic ATP depletion (Piper 2004) (Fig. 1.1, fig. 1.2).

- Intracellular acidosis develops due to anaerobic glycolysis and accumulation of lactate (Cobbe 1980).
- To combat intracellular acidosis the Na^+/H^+ exchanger extrudes hydrogen ions in exchange for sodium ions (Lazdunski 1995) and the $\text{Na}^+/\text{HCO}_3^-$ symporter transports sodium and bicarbonate into the cell. This results in an accumulation of cytosolic sodium. Neubauer (1987) reported that $[\text{Na}^+]_i$ increased during ischemia in proportion to the reduction of intracellular pH.
- Activity of the Na^+/K^+ ATP-ase is inhibited rapidly after the onset of ischemia due to a decrease in extracellular pH and ATP depletion. The Na^+/K^+ ATP-ase is exquisitely sensitive to extracellular pH (Piwnicka-Worms 1985, Lazdunski 1985) and failure of the Na^+/K^+ ATP-ase results in the inability of the cell to extrude sodium. Large increases in intracellular sodium ion concentration during ischemia do not occur without the inhibition of Na^+/K^+ ATP-ase activity (Hoerter 1986).
- Intracellular sodium ion concentrations are further increased due to the fact that sodium influx through the fast sodium channel ($3 - 6 \mu\text{g/g/min}$)

(Cohen 1982) during the upstroke of the action potential, is not affected during the first 10 – 20 minutes of ischemia (Sperelakis 1988). The initial upstroke of the action potential during ischemia remains unchanged until the heart reaches quiescence (Tani 1990).

- Marked elevation of intracellular sodium ion concentration during ischemia has been confirmed with nuclear magnetic resonance (Pike 1988, Balschi 1985, Van Echteld 1991), and with ion selective microelectrodes (Wilde 1986).
- The cytosol is now sodium overloaded and the cell membrane depolarized. This causes the $\text{Na}^+/\text{Ca}^{2+}$ exchanger to operate in reverse mode, (extruding sodium ions in exchange for calcium ions) leading to an ***absolute increase in the total intracellular calcium*** ($[\text{Ca}^{2+}]_i$) (Tani 1990, Silverman 1994).
- Reversal of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is the major source of calcium overload **during ischemia** (Tani 1990).
- The $\text{Na}^+/\text{Ca}^{2+}$ exchanger reverses during hypoxia/ischemia once ATP depletion has occurred, and is not rate limiting for intracellular calcium loading after complete ATP depletion. Pharmacological inhibition of the exchanger at this point will reduce calcium loading and improve cell survival later at reoxygenation (Ziegelstein 1992, Hagny 1992).
- Sarcolemmal and sarcoplasmic reticulum calcium ATP-ase fail (ATP depleted) (Chemnitz 1985), resulting in an inability to extrude excess calcium.

- Steenbergen (1987) reported that cytosolic free calcium ion concentration started to increase after 6 minutes of ischemia and reached $3\mu\text{M}$ after 9 – 15 minutes.
- Cell outcome following hypoxia/ischemia is tightly correlated with the absolute level of $[\text{Ca}^{2+}]_i$ achieved at the end of the ischemic period prior to reperfusion. Single rat myocytes will hypercontract immediately if $[\text{Ca}^{2+}]_i$ exceeds $5\mu\text{M}$ just prior to reperfusion. However if the cell is reoxygenated before $[\text{Ca}^{2+}]_i$ reaches 2 – 3 μM , the cell will relengthen and survive (Snowdone 1985, Allshire 1987).

1.1.2.2 Reperfusion (reoxygenation) (Fig. 1.1, Fig. 1.2).

On reperfusion, extracellular fluid is quickly replaced by perfusate with a normal pH, this results in rapid reactivation of Na^+/K^+ ATP-ase. In addition, restoration of mitochondrial oxidative phosphorylation, recovery from tissue acidosis and washout of ischemic metabolites on reperfusion together with an increased intracellular sodium ion concentration, accelerates Na^+/K^+ ATP-ase function (Tani 1990). The Na^+/K^+ ATP-ase accounts for most of the decline in intracellular sodium during reperfusion (Sejersted 1988).

Due to the recovery of oxydative phosphorylation, the ATP necessary for the functioning of the contractile machinery and for the functioning of Ca^{2+} ATP-ase and Na^+/K^+ ATP-ase is immediately available.

Contractile function is activated and the cell membrane is repolarized by Na^+/K^+ ATP-ase before the excess calcium has been extruded (Piper 2003). Besides repolarizing the cell membrane the functioning sodium pump (Na^+/K^+ ATP-ase) also restores the $[\text{Na}^+]_i : [\text{Na}^+]_o$ gradient. This gradient is the major driving force for calcium efflux via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. The $\text{Na}^+/\text{Ca}^{2+}$ exchangers switches again to the forward mode, extruding calcium in exchange for sodium.

Extrusion of calcium from the cell is dependant on:

- The plasma membrane calcium pump (Ca^{2+} ATP-ase) which has a high affinity for calcium, but a limited capacity and can be overwhelmed.
- The $\text{Na}^+/\text{Ca}^{2+}$ exchanger has a greater capacity (10 times that of the sarcolemmal Ca^{2+} ATP-ase) (Carafoli 1985, Barry 1985), but is dependant on sodium movement. It transports calcium outwards during diastole and inwards during systole.

Thus the lowering of total intracellular calcium is not immediate.

During ischemia, due to ATP depletion and acidosis, the sarcoplasmic reticulum and mitochondria cannot take up the excess calcium ions accumulating in the cytosol (Mandel 1982, Williams 1983). On reperfusion however, ATP production is restored and intracellular acidosis is corrected, this will allow the sarcoplasmic reticulum and mitochondria to accumulate calcium ions (Meno 1984, Nakanishi 1982, Williams 1983). Once the sarcoplasmic reticulum is full

it starts releasing calcium leading to oscillatory cytosolic calcium concentration elevations (Siegmund 1997, Schäfer 2001). Calcium spontaneously released from a calcium overloaded sarcoplasmic reticulum may induce adjacent sarcoplasmic reticulum calcium release via the calcium release channel, resulting in a propagating wave of increasing $[Ca^{2+}]_i$. This triggers calcium overload induced hypercontracture (Berlin 1989, Wier 1991, Williams 1992).

The explanation for the hypercontracture caused by the excessive cytosolic calcium oscillations during the first few moments of reperfusion is as follows:

Rapid beat to beat changes in cardiac performance (contractility) are brought about largely by variations in the amount of calcium bound to troponin C. Maximum isometric tension is generated when all available troponin C molecules are bound to calcium (Katz 2001 b). The most important determinant of the number of troponin C molecules to be bound, is the amount of calcium released by the sarcoplasmic reticulum during the excitation/contraction coupling (Katz 2001, b).

All striated muscle myocytes have a transverse tubular system (t-tubules) that opens into the extracellular space. The composition of the fluid in the t-tubules is identical to that of the extracellular fluid: high calcium and sodium concentration and low potassium concentration. The t-tubule membrane is an extension of the sarcolemma and its purpose is to carry the action potentials deep into the myosite (Katz 2001; c).

Although electrical depolarization of the cell is essential for rapid activation, muscle contraction depends on **calcium** which must bind to troponin C. There is a massive inward concentration and electrical gradient for calcium. The calcium concentration in the extracellular fluid is $> 1\text{mM}$ which is 5000 times higher than the calcium concentration in the cytosol of the resting heart ($0,2\mu\text{M}$). The calcium concentration required to saturate troponin C is less than $10\mu\text{M}$ (Kretsinger 1977).

The initial event for excitation of the myocyte is a brief inward (depolarizing) sodium current. This leads to a partial depolarization of the cell membrane, enough to open **voltage gated calcium channels**. A small amount of calcium enters the cell, following the electrochemical gradient and binds to the ryanodine receptor (calcium release channel) on the sarcoplasmic reticulum. This triggers a much larger calcium release from the sarcoplasmic reticulum (Katz 2001; c). The normal influx of calcium ions via the voltage gated calcium channels is $0,1 - 0,3 \mu\text{mol/g/min}$ (1nmol /g/beat) (Lullman 1983).

The most important voltage gated calcium channels are the L-type calcium channels. Opening of these channels is prolonged, and they are also known as dihydropyridine receptors as they bind calcium channel blocking drugs (Katz 2001; c).

There are two calcium cycles important to the cardiomyocyte (Katz 2001; d):

1. Extracellular cycle:

Calcium moves across the sarcolemma between the extra cellular fluid and the cytosol.

2. Intracellular cycle:

Calcium moves between the cytosol and the stores in the sarcoplasmic reticulum.

The extracellular cycle triggers the intracellular cycle and the intracellular cycle provides the calcium needed to bind troponin C. The sarcoplasmic reticulum is the primary source and sink controlling intracellular calcium ion transients that mediate myofilament interaction (Fabiato 1983, Wier 1990, Barry 1993). The sarcoplasmic reticulum calcium release determines the cytosolic calcium concentration. The higher the cytosolic calcium concentration during each cycle, the stronger the contraction.

Calcium release channels in the cardiomyocyte sarcoplasmic reticulum (ryanodine receptors) are highly regulated: (Katz 2001; d)

- Increased entry of extracellular calcium through the L-type plasma membrane calcium channels increases calcium release from the sarcoplasmic reticulum.

- The L-type plasma membrane calcium channels regulate calcium release by the sarcoplasmic reticulum, as well as sarcoplasmic reticulum calcium content (Barry 1993, Beukelmann 1988, Sperelakis 1988).
- **Most importantly:** The larger the **size** of the **calcium store** in the **sarcoplasmic reticulum**, the **more** calcium is released.
- ATP exerts an allosteric effect that promotes channel opening.
- Xanthines (caffeine) initiate calcium release.
- Acidosis inhibits channel opening.

In ***skeletal muscles*** the intracellular sarcoplasmic reticulum calcium stores are large and release so much calcium that all the potential interacting sites between actin and myosin are activated (all troponin C is bound). Maximum tension is developed on every contraction. This is an all or nothing response.

Cardiomyocytes have the ability to vary contractility, therefore calcium fluxes across the sarcoplasmic reticulum are regulated. Under normal conditions the sarcoplasmic reticulum calcium stores are variable, but much smaller than those of skeletal muscles. A varying, but smaller amount of calcium is released, and fewer troponin C molecules are bound. This allows the contractile response to be graded (Katz 2001; d).

However, in the first few moments of reperfusion all the excess calcium accumulated in the cytosol during ischemia is taken up by the sarcoplasmic reticulum, resulting in a calcium overloading of the sarcoplasmic reticulum.

Every time the sarcolemma depolarizes and the voltage gated calcium channels open, there is an inward movement of a relatively small amount of calcium (extracellular cycle) which triggers the intracellular cycle. Because of the large sarcoplasmic reticulum calcium store early in reperfusion, a large amount of calcium is released and a large proportion of troponin C molecules are bound leading to hypercontracture. Furthermore, the maximal rate of calcium ion influx through the L-type voltage gated sarcolemmal calcium channels during early reperfusion is $0,5\mu\text{mol/g/min}$, 2 – 5 times more than normal (Tani 1988, 1989). Therefore these channels may account for significant increases in cytosolic calcium ion concentration during early reperfusion, and induce further accumulation of calcium ions by the sarcoplasmic reticulum.

In support of the importance of the role of sarcoplasmic reticulum calcium release in hypercontracture and myocardial stunning, Mitchell (1993), using isolated rat hearts subjected to 20 minutes of ischemia followed by 40 minutes of reperfusion, reported that when sarcoplasmic reticulum calcium release on reperfusion was inhibited with sodium dantrolene, myocardial stunning was attenuated. Systolic and diastolic function was improved as compared to controls. It is important to note that dantrolene most likely inhibits sarcoplasmic calcium ion release by interfering with the coupling of the sarcoplasmic reticulum to sarcolemmal depolarization (it prevents the extracellular cycle from triggering the intracellular cycle). (Honerjager 1983, Hiraoka 1985). Ryanodine (a direct sarcoplasmic reticulum calcium channel inhibitor) administered either before

ischemia or during early reperfusion also attenuates stunning in the isolated rat heart (Du Toit 1992).

In conclusion, hypercontracture and stunning on reperfusion are the result of intracellular calcium accumulation during ischemia, and further calcium accumulation and rapid calcium cycling on reperfusion (Fig. 1.3).

Reperfusion induced hypercontracture leads to a marked rise in end-diastolic pressure and ventricular wall stiffness during the first few minutes of reperfusion. (Piper 2004).

Cell death occurs almost exclusively in the first minutes of reperfusion and hypercontracture is the primary cause of cardiomyocyte necrosis (contraction band necrosis) in this early stage. Thereafter, for the next few hours, other causes lead to a further increase in cell death, either by necrosis or apoptosis (Piper 2003, Humphrey 1986).

The first few minutes of reperfusion represent a window of opportunity for cardio protection: (Piper 2004).

- Reduction of sarcoplasmic reticulum calcium oscillations by:
 - Inhibition of calcium influx into cells.
 - Inhibition of calcium release by the sarcoplasmic reticulum (Siegmond 1997, Ladilov 1995, Schäfer 2000).

Inhibition of the contractile machinery until intracellular calcium concentration has normalized by:

Acidosis (Ladilov 1995, Schäfer 2000).

Inhibition of troponin I (protein kinase G)

(Garcia-Dorado 1992, Siegmund 1991).

The later reperfusion injury – during the first two hours of the reflow, includes the following pathological processes: (Piper 2003, Padilla 2000, Schluter 1994).

1. The endothelium of blood vessels becomes permeable leading to interstitial edema.
2. Endothelial cells are activated, express adhesion molecules, release cytokines and stop producing nitric oxide.
3. This leads to adherence, activation and accumulation of neutrophils and monocytes in the myocardium.
4. These activated leucocytes release oxygen free radicals and proteolytic enzymes which further damage myocytes and endothelial cells.
5. Adherent leucocytes, in addition to tissue contracture and interstitial edema, plug blood vessels causing slow/no reflow phenomena.

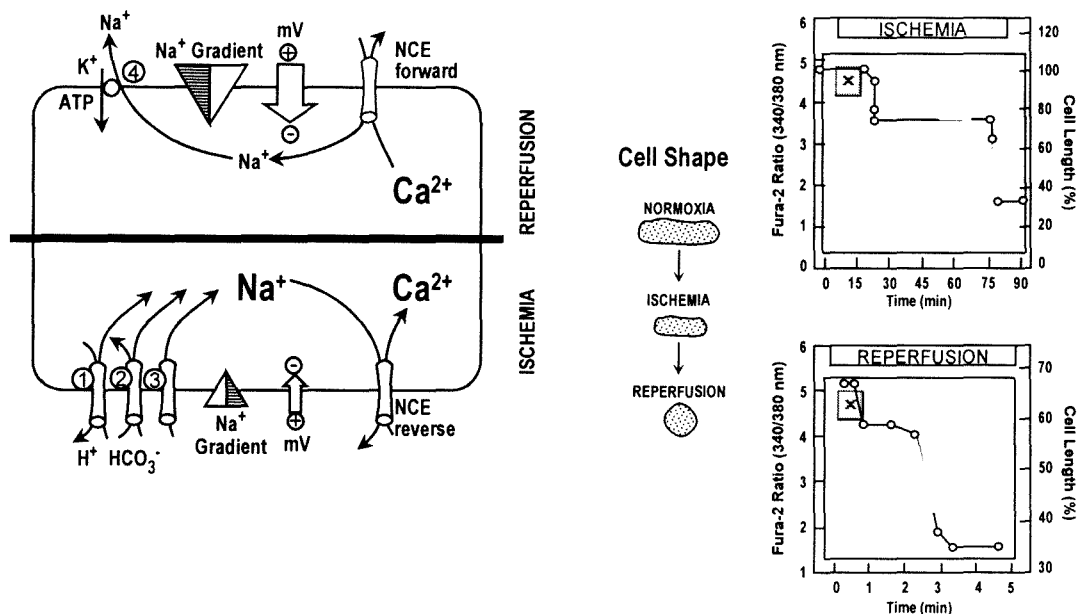


Fig. 1.1

Left: During ischemia the cardiomyocyte accumulates sodium via the sodium/hydrogen exchanger (1) , the sodium/bicarbonate symporter (2) and failure of the sodium/potassium ATPase (4) Due to increased intracellular sodium and the membrane depolarization, the sodium/calcium exchanger switches to *reverse mode*. This results in intracellular calcium accumulation.

At the onset of reperfusion the energy recovery reactivates the sodium/potassium ATPase (4). This restores the sodium gradient and reduces the intracellular sodium. The cell membrane is repolarized. The sodium/calcium exchanger switches to *forward mode* and extrudes the accumulated calcium.

Right: Typical changes in cytosolic calcium and cell length. in a single cardiomyocyte during ischemia and reperfusion. Cells elongated in normoxia , rigor shortened during ischemia and hypercontracted on reperfusion. During ischemia intracellular calcium rises ; at the onset of reperfusion intracellular calcium declines (as the calcium is taken up by the SR), but immediately starts to oscillate. During these oscillations extensive contracture develop.

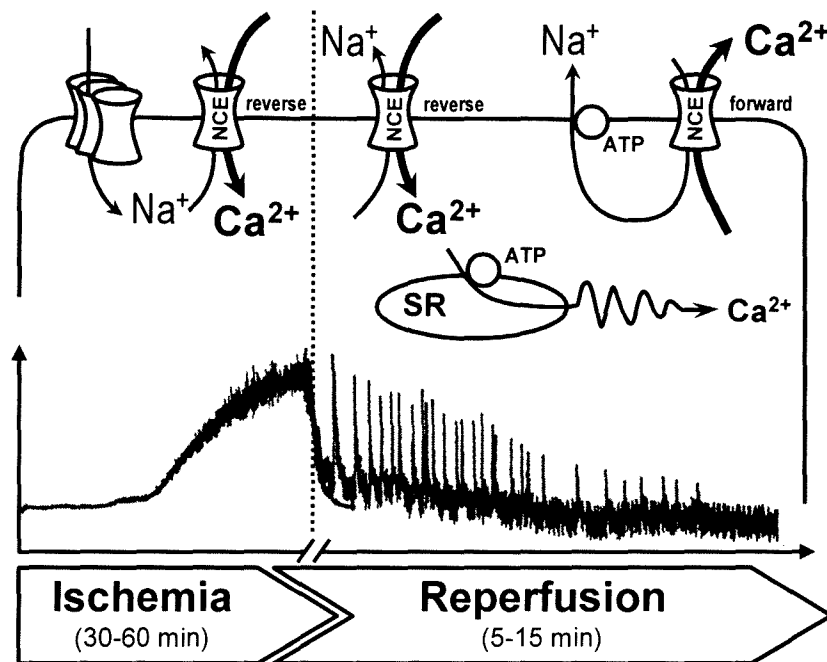


Fig. 1.2

Cytosolic calcium overload during ischemia due to reverse mode operation of the sodium/calcium exchanger as a result of increased intracellular sodium and membrane depolarization. The intracellular sodium is increased due to sodium/hydrogen exchange, sodium/bicarbonate symport and the failure of sodium/potassium ATPase. The latter also causes membrane depolarization. At the onset of reperfusion, sodium/potassium ATPase is restored, intracellular sodium decreases; the membrane repolarized and the SR sequesters excess cytosolic calcium. Spontaneous release and reuptake of calcium by the sarcoplasmic reticulum leads to cytosolic oscillations of calcium

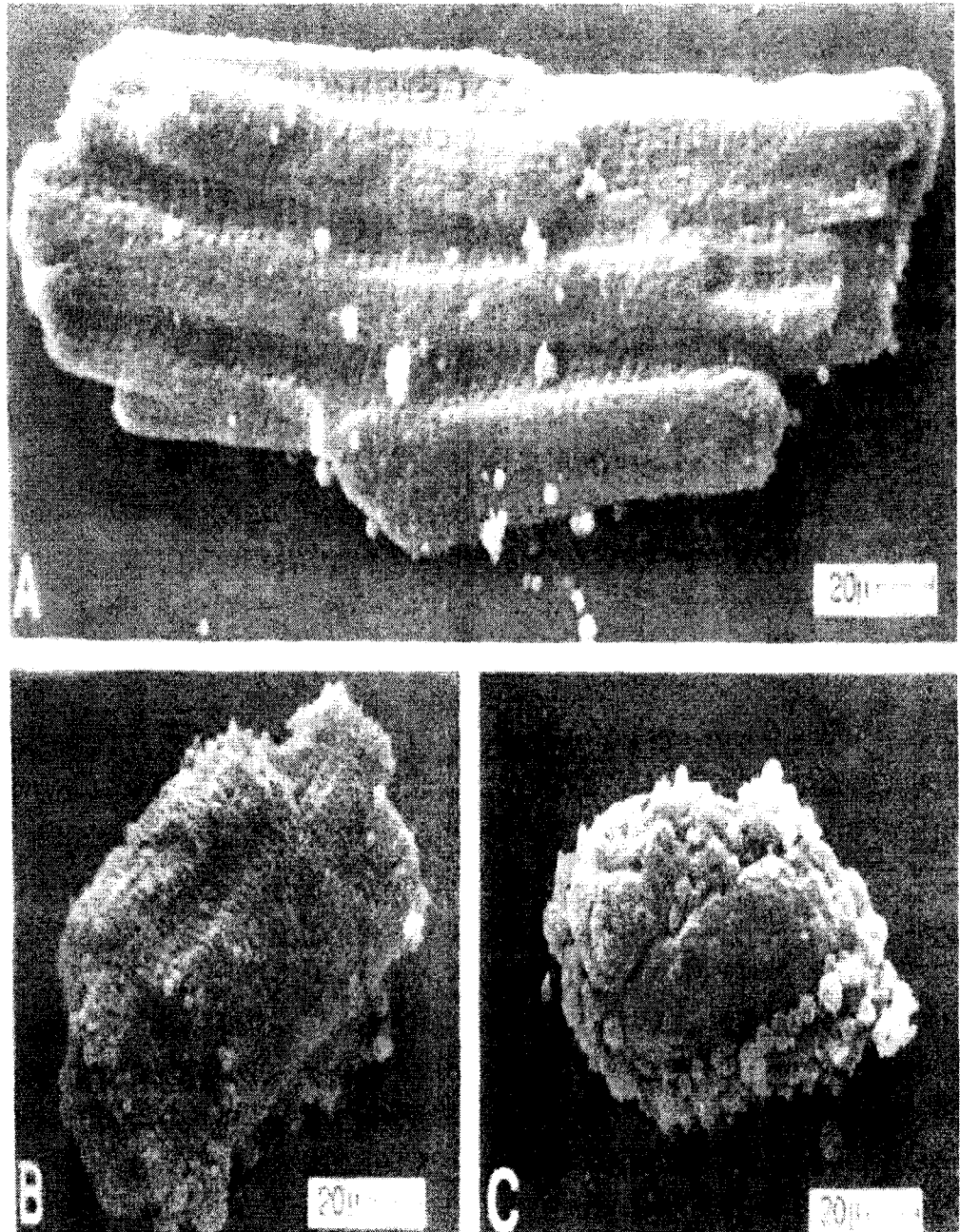


Figure 1.3

Electron micrographs of single cardiomyocytes:.

A: During normoxia.

B: During anoxia following rigor development.

C: Reoxygenation following hypercontracture. Note the numerous spherical blebs.

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1.1.3 Secondary effects of calcium in the reperfusion injury.

Because this does not relate to the primary aim of this thesis (see section 1.4) it will be dealt with in relative brevity, and only for completeness sake.

1.1.3.1 Mitochondrial permeability transition pore (MPTP) opening.

The main function of mitochondria under normal conditions is ATP production through oxidative phosphorylation. The mitochondrial inner membrane is usually impermeable to all, but a few selected ions.

Under certain conditions latent mechanisms within the mitochondria can be activated. The mitochondria will then be converted from organelles that support the life of the cell to those that actively induce both apoptotic and necrotic cell death. This conversion is mediated by the opening of a nonspecific pore in the mitochondrial inner membrane, known as the mitochondrial permeability transition pore (MPTP). This pore normally remains closed, but can open under conditions of cellular stress, with dire consequences (Halestrap 1999).

During reperfusion mitochondrial calcium overload (increased mitochondrial matrix calcium concentration) is the key factor responsible for MPTP opening, and the extent of MPTP opening is a critical determinant of the reperfusion injury (Halestrap 2004). At the onset of reperfusion mitochondria are again able to respire and generate a membrane potential to drive ATP synthesis, but this also results in a rapid accumulation of calcium within the mitochondria, leading to

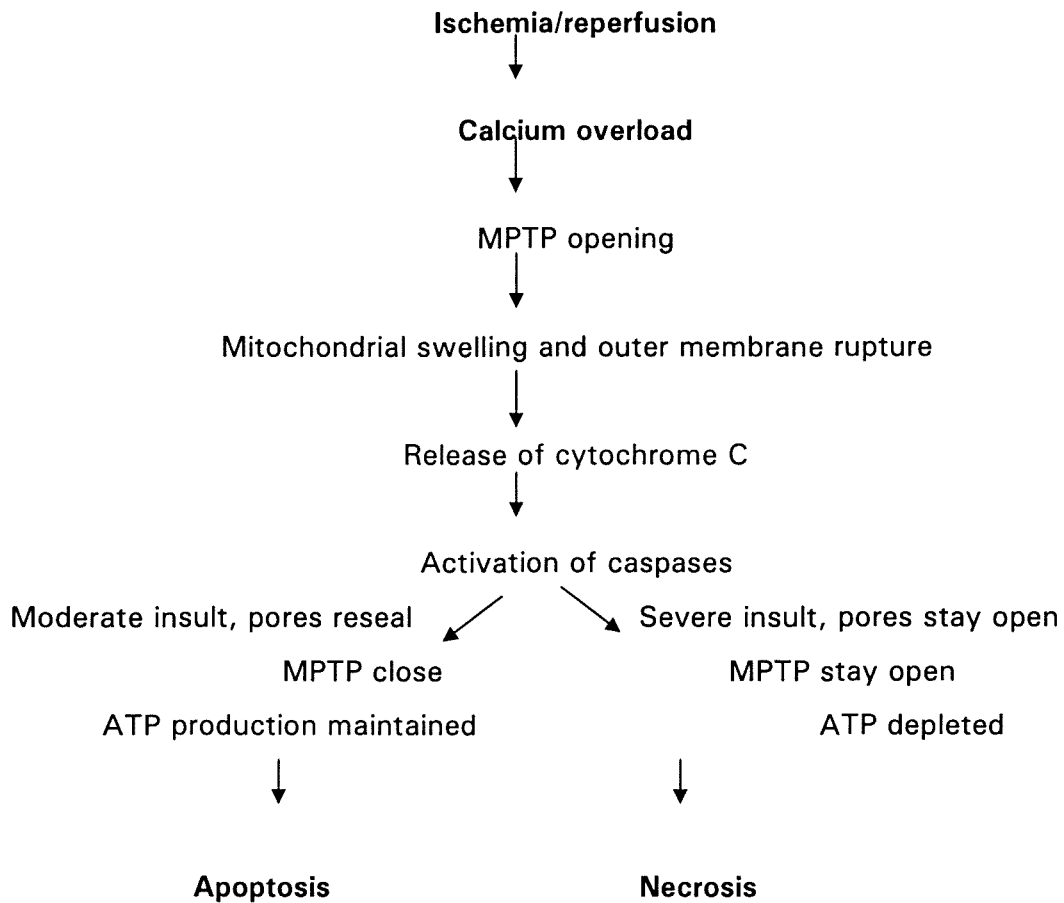
calcium overload. The high intramitochondrial phosphate, oxidative stress and low adrenergic nucleotide concentrations, present at this time, all potentiate the effect of calcium (Silverman 1994).

Calcium triggers a conformational change that causes the opening of the MPTP. When the MPTP opens, the permeability barrier of the inner membrane becomes disrupted with two major consequences (Halestrap 1998):

- Small molecular weight solutes can now move freely across the membrane, but proteins cannot, and as a result they exert a colloid osmotic pressure, which causes the mitochondria to swell. The inner membrane expands easily, but the outer membrane ruptures, spilling proteins such as cytochrome C, which triggers apoptosis.
- The inner membrane becomes freely permeable to protons. This leads to the uncoupling of oxidative phosphorylation and the mitochondria convert from ATP synthesis to ATP hydrolysis.

If the pores remain open these changes will cause irreversible damage to the cell resulting in necrotic death. Even if the pores close, mitochondrial swelling and outer membrane rupture may be sufficient to set the apoptotic cascade in motion (Crompton 1999).

These pathways are summarized as follows:



1.1.3.2 Gap junction mediated intracellular communication (GJMIC).

GJMIC is essential in electrical impulse propagation in the heart and altered GJMIC may lead to arrhythmias (Garcia Dorado 2004). Gap junctions are specialized membrane areas that contain tightly packed channels that allow direct connection of the cytoplasm of two adjacent cells (Harris 2001; Sosinsky 2000).

During reperfusion GJMIC mediates cell to cell propagation of hypercontracture and cell death (Garcia Dorado 1989).

Most of the cell death induced by transient ischemia occurs during the first few minutes of reperfusion due to contraction band necrosis (Humphrey 1986). Hypercontracted, dead cardiomyocytes are connected to other dead myocytes in well defined areas of contraction band necrosis. Cell to cell propagation of hypercontracture is dependent on induction of a rapid increase in intracellular calcium concentration in the adjacent cell (García Dorado 2000).

The rapid rise in intercellular calcium concentration in the second cell is probably caused by passage of sodium from the hypercontracting cell through the gap junction and a subsequent reverse mode operation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in the second cell (Ruiz-Meana 1999).

Propagation stops when an adjacent less injured cell is able to cope with sodium and calcium influx efficiently and so prevents severe calcium overload.

1.1.3.3 Apoptosis.

Apoptosis indicates cell death and removal without activation of an inflammatory process. Caspase activation is required (Hunot 2001).

Prolonged ischemia causes necrosis while reperfusion enhances apoptosis (Eefting 2004; Gottlieb 1994). Mitochondrial calcium overload on reperfusion triggers opening of MPTP which results in the release of cytochrome C which in turn activates caspase (Borutaite 2003).

Ion channels play an important role in calcium overload and hypercontracture (Piper 2003). There is no debate on the role of membrane channel blockers in the prevention of calcium overload and hyper contracture; the question is whether apoptosis will also be reduced.

Blocking calcium fluxes through L-type calcium channels does seem to inhibit apoptosis and reduce infarct size; as Gourine (2001) demonstrated with the use of clevidipine in the porcine model and Jang (2003) with the use of nicardipine in the canine model.

1.1.3.4 Decreased nitric oxide (NO) production.

Endothelial NO is formed from L-arginine by the constitutive form of the enzyme NO-synthase. NO is an important regulator of vascular tone, prevents platelet and leukocyte adherence and is a scavenger of super oxide (Moncada 1999).

During myocardial ischemia and reperfusion endothelial dysfunction occurs during early reperfusion, and is an important event in the development of ischemia-reperfusion injury (Tsao 1990).

Early reperfusion results in impaired endothelium dependant vasodilatation which is the result of reduced NO formation or rapid inactivation of NO by oxygen-derived free radicals (Lefer 1997). Augmentation of NO levels during reperfusion

preserved endothelial function, reduced neutrophil accumulation and decreased myocardial injury (Lefer 1996). Thus NO appears to be a crucial factor mediating protection against the ischemia-reperfusion injury.

Calcium antagonists can release NO from coronary micro vessels (Zhang 1998), reduce infarct size and preserve endothelial function by maintaining the local bio availability of NO (Gourine 2001).

1.1.3.5 Production of reactive oxygen species.

Polymorphonuclear leucocytes are a major source of toxic oxidants in vivo, causing tissue injury in ischemia and reperfusion (Feng 1996).

Calcium ions are a key mediator in the regulation of oxidant formation by polymorphonuclear leucocytes and in maintaining optimal function of these cells (Romeo 1975). The production of superoxide anions by stimulated neutrophils is augmented when calcium ions are available in the extracellular environment (Goldstein 1975). Pharmacological stimulation of calcium ion movement across the neutrophil membrane produces a burst of metabolic activity culminating in the release of oxidants (Korchak 1978).

Calcium antagonists can suppress the capacity of neutrophils to produce oxidants, even though drug sensitive calcium channels have not been demonstrated in neutrophils (Pennington 1986). Thus the ability of these drugs

to inhibit oxidant production seems to be attributable to some mechanism other than their calcium channel blocking properties.

1.2 Physiology

1.2.1 Voltage gated ion channels.

1.2.1.1 Sodium fluxes and sodium channels.

1.2.1.2 Calcium currents and calcium channels.

1.2.1.3 Potassium channels.

1.2.2 The plasma membrane.

1.2.2.1 The plasma membrane calcium pump (Ca^{2+} -ATP-ase).

1.2.2.2 The sodium calcium exchanger.

1.2.2.3 The sodium pump (Na^+/K^+ -ATP-ase).

1.2.2.4 The sodium-hydrogen exchanger.

1.2.3 The sarcoplasmic reticulum (SR)

1.2.3.1 Structure of sarcoplasmic reticulum.

1.2.3.2 Calcium release channels (ryanodine receptors).

1.2.3.3 Calcium pump of the sarcoplasmic reticulum (SERCA).

1.2.4 The mitochondrion.

1.2.5 Calcium as intracellular messenger.

1.2.6 Calcium cycles.

1.2 Physiology.

1.2.1 Voltage gated ion channels (Katz 2001; g).

Ion movement through these channels is down electrochemical gradients and no energy expenditure is required. These channels are membrane protein complexes made up of several subunits: $\alpha 1$, $\alpha 2$, β , γ , δ .

Voltage gated ion channels contain ion selective pores that favour the passage of the single ion species.

The channel has three states:

Closed (resting)

Open

Closed (inactive/refractory)

The same depolarizing signal that opens the resting channel causes it to close in a refractory state (closing takes slightly longer). Reactivation in the case of sodium and calcium channels occurs when the membrane is repolarized by outward potassium currents (Fig. 1.4).

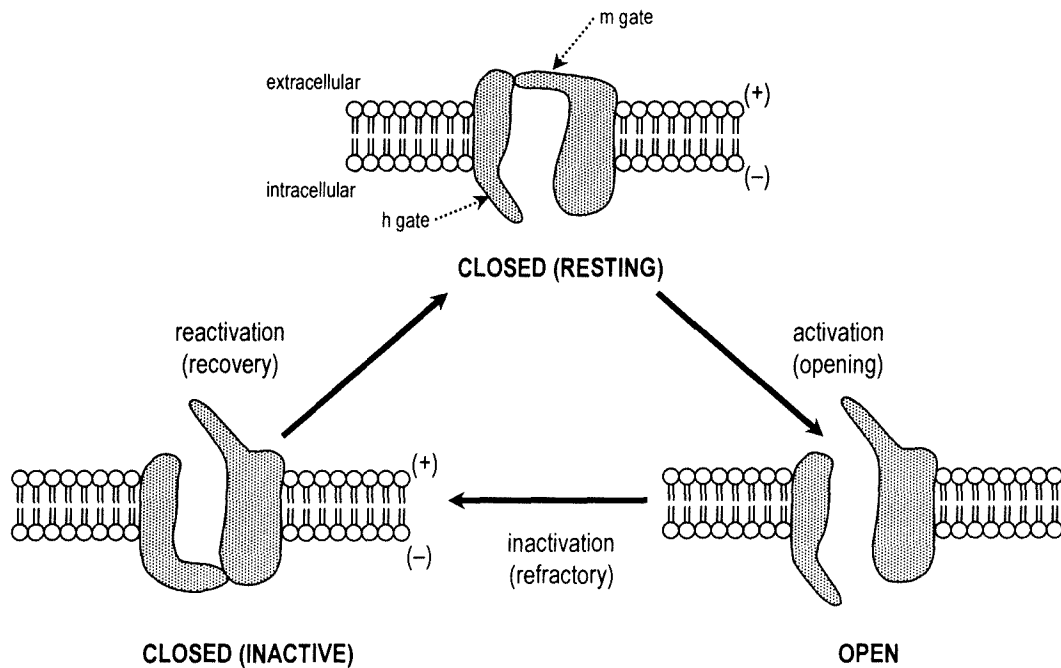


Fig. 1.4

The three states of the voltage gated ion channel. In the refractory (inactive) state, it cannot be reopened. In the resting (recovered) state, the channel can be opened by membrane depolarization.

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1.2.1.1 Sodium fluxes and sodium channels.

The sodium currents that generate initial upstroke of the cardiac action potential serve two major roles (Katz 2001; c).

1. To propagate the action potential.
2. The large inward depolarizing sodium current opens the plasma membrane L-type voltage gated calcium channels. Calcium influx through these channels in turn triggers calcium release from the internal stores.

In 1952 Hodgkin and Huxley described two different gating mechanisms in the sodium channel; m and h. Opening the m gate activates (opens) the channel. Closing the h gate inactivates (closes) the channel (Fig. 1.4).

Depolarization opens (activates) the m channel leading to an inward current of sodium. The same depolarization initiates a slower process that closes the h gate so inactivating the channel. Sodium ions can enter only when both gates are open thus only in the brief time between the opening of the m gate and closing of the h gate.

The h gate can only be reopened after repolarization has returned the membrane potential to its resting level. Then the cycle can be reactivated (Katz 2001 g).

Influx of Na^+ through the fast Na^+ channel is 20 – 40 nmol/g/beat or 3 – 6 $\mu\text{mol/g/min}$ (Cohen 1992).

1.2.1.2 Calcium currents and calcium channels.

Calcium channels become activated and inactivated in the same way as sodium channels but in the case of calcium, the transitions occur at more positive membrane potentials and more slowly; therefore, calcium channels have been termed slow channels. Opening of the voltage gated calcium channels in the plasma membrane allows relatively small amounts of calcium to enter the cell, driven by a large electrochemical gradient. (Extracellular $[Ca^{2+}] = 1\text{mM}$; cytosolic $[Ca^{2+}]$ during diastole = $0.2\text{ }\mu\text{M}$ hence the 5000 fold gradient) (Katz 2001 h).

The most important calcium channel is the L-type calcium channel. It's opening is prolonged and it is also called the dihydropyridine receptor because it binds with high affinity to this class of calcium channel blocking drugs (Katz 2001 c). In normal myocytes calcium ion influx through this channel is less than 1nmol/g/beat or $0,1 - 0,3\text{ }\mu\text{mol/g/min}$ (Lullman 1983). This amount of Ca^{2+} must be extruded each cycle by mainly the Na^+/Ca^{2+} exchanger (Wier 1990).

The calcium that enters the cell in this way has five functions (Katz 2001 c):

1. It causes further depolarization of the cell.
2. It activates potassium channels thus initiating repolarization.
3. It provides calcium for binding to troponin C (only a small amount).

4. It fills the stores in the sarcoplasmic reticulum, maintaining contractility.
5. **Most importantly it triggers calcium release from the sarcoplasmic reticulum, thus activating contraction.**

Sarcolemmal voltage gated slow calcium ion channels regulate sarcoplasmic calcium content and sarcoplasmic reticulum calcium release (Barry 1993, Beukelman 1988, Sperelakis 1988) and Ca^{2+} entry across the cell membrane is the major regulatory site for the force of contraction (Sperelakis 1988).

The Treppe effect can be explained as follows: More frequent opening of voltage gated calcium channels leads to more calcium entering the cell. This has two consequences (Katz 2001f):

1. Larger sarcoplasmic reticulum calcium stores.
2. More frequent triggering of calcium release from the sarcoplasmic reticulum.

The net result is an increase of tension development.

Post extrasystolic potentiation is caused when a premature depolarization increases calcium entry via the voltage gated calcium channels and so increases the calcium stores in the sarcoplasmic reticulum. The premature depolarization prolongs the opening time of the voltage gated calcium channel. All the extra calcium is taken up by the sarcoplasmic reticulum thus adding to the store, and so to the tension of subsequent contractions (Katz 2001 f).

Cyclic AMP: β -adrenergic agonists and PDE inhibitors both increase cyclic AMP (cAMP). cAMP-dependant protein kinases cause phosphorylation of plasma membrane voltage gated calcium channels. Phosphorylation increases the probability of the calcium channel being open, leading to increased calcium entry and so a positive inotropic effect (Katz 2001 f).

Calcium channel blocking drugs modify L-type calcium channel opening so as to reduce calcium entry. This results in:

1. Smaller sarcoplasmic reticulum calcium stores.
2. Diminished calcium triggered calcium release from the sarcoplasmic reticulum.

Both of the above lead to a negative inotropic effect (Katz 2001 f).

Despite their quite different chemical structures, verapamil, nifedipine, diltiazem and bepridil all depress Ca^{2+} influx through the slow (L-type) channels by a direct action on the channel itself (Vogel 1979, Molyvdas 1983). Binding of a drug to a receptor within an ion channel is influenced by the state of the channel, which is determined by the membrane potential. Binding of the calcium antagonists is approximately 1000 times stronger to inactivated channels than to resting channels (Sanguinetti 1984). During ischemia the sarcolemma is depolarized and the calcium channels are all in the inactive state.

Voltage gated calcium channels are membrane protein complexes made up of several subunits: $\alpha 1$, $\alpha 2$, β , γ , δ . Two forms of calcium channel $\alpha 1$ peptide are found in muscle. The larger molecule is found in L-type channels responsible for the depolarizing calcium currents that create the plateau of the cardiac action potential. (The smaller molecule is the voltage sensor that opens calcium release channels of skeletal muscle sarcoplasmic reticulum).

L-type calcium channels are the most abundant calcium channels in cardiomyocytes and vascular smooth muscle cells. The long lasting depolarizing currents carried by these channels are responsible for the plateau of the cardiac action potential (Katz 2001; g).

L-type calcium channels are located in the transverse tubular system (t-tubules) very close to the calcium release channels of the sarcoplasmic reticulum (ryanodine receptors). This is important for calcium triggered calcium release. T-type calcium channels are not concentrated in the t-tubules (Bogdanov 1995).

The $\alpha 1$ subunit of the L-type calcium channel is the substrate for cAMP-dependant protein kinase-catalyzed phosphorylation. This increases the chance of the channel being open and increases the number of available channels (Cachelin 1983), thus causing a positive inotropic effect. Phosphorylation therefore, increases the slow inward Ca^{2+} current during the action potential, thus elevating $[\text{Ca}^{2+}]_i$ directly (by increasing the mean open time and number of L-type channels) and indirectly (by Ca^{2+} induced Ca^{2+} release from the

sarcoplasmic reticulum), and thus increasing the force of contraction (Sperelakis 1988, Bean 1984).

In addition to the closed state (mode 0), L-type calcium channels can be open in two states: Brief openings (mode 1) and long lasting opening (mode 2). Both modes are natural states of the channel (Bean 1990). cAMP induced phosphorylation initiated by β adrenergic agonists favours the appearance of mode 2 as do strong depolarizations (Yue 1990, Pietrobon and Hess 1990).

Ca^{2+} influx via the L-type Ca^{2+} channel is inhibited by cGMP-mediated phosphorylation of a protein involved in L-type Ca^{2+} channel function (Fischmeister 1987). Acetylcholine depresses Ca^{2+} influx via the L-type channels by reversing cAMP elevation produced by various agonists (Wahler 1986, Josephson 1982), and by elevation of cGMP levels (MacLeod 1986).

The myocardial slow (L-type) Ca^{2+} channels are selectively blocked by acidosis (Chesnais 1975, Vogel 1977). As pH decreases $[\text{Ca}^{2+}]_i$ decreases, and contractions become depressed and abolished as a function of the degree of acidosis. Due to the fact that acidosis has little effect on the normal fast action potential, (only Ca^{2+} channels are blocked), excitation – contraction uncoupling occurs (Sperelakis 1988).

Due to the fact that Ca^{2+} flux through the L-type Ca^{2+} channels is modulated by pH and ATP availability, the channels are inactivated within 5 – 15 minutes after the induction of myocardial ischemia (Sperelakis 1988).

The T-type calcium channels are inactivated more rapidly than the L-type calcium channels. Once the membrane is depolarized the T-type channels quickly close and become refractory (Katz 2001 g).

T-type calcium channels also have less conductance and so admit less calcium than L-type channels. The threshold of T-type channels is lower so they are opened by smaller depolarizations than L-type channels (Nilius 1985, Tsien 1998). This explains their role in generating pacemaker potentials in the SA node.

However in atria, ventricles and the His-Purkinje system, the transient currents carried by the T-type calcium channels occur at the same time as the much larger sodium currents so that they play almost no role in membrane depolarization (Arreola 1991).

The ability of T-type calcium channels to slowly admit small amounts of calcium leads to the generation of prolonged calcium signals that regulate cell growth and proliferation (Katz 2001 g).

1.2.1.3 Potassium channels.

These are the most primitive of the voltage gated channels, they are also the most complex. The diversity of potassium channel structure is the reason why so many types of potassium channels are found in the human heart (Katz 2001g). Further discussion of the potassium channels is beyond the scope of this thesis.

1.2.2 The plasma membrane.

There are two mechanisms by which the cardiomyocyte extrudes calcium across the cell membrane.

1. ATP dependant calcium pumps (Ca^{2+} -ATP-ase).
2. $\text{Na}^+/\text{Ca}^{2+}$ -exchanger.

The $\text{Na}^+/\text{Ca}^{2+}$ -exchanger has a greater capacity than the Ca^{2+} -ATP-ase pump, but a lower affinity for calcium. The Ca^{2+} -ATP-ase is the primary mechanism for calcium extrusion but it is easily overwhelmed thus requiring an additional high capacity system namely the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Katz 2001 c).

1.2.2.1 The plasma membrane calcium pump (Ca^{2+} ATP-ase).

Like all the ATP-dependant cation pumps:

- It uses energy derived from hydrolyses of ATP to actively transport ions.
- It is an intrinsic membrane protein with 10 transmembrane segments.
- It contains ATP binding, phosphorylation and cation-binding sites in the cytosol.

In its basal state the plasma membrane Ca^{2+} -ATP-ase is inhibited by a large C-terminal loop in the cytosol. The inhibitory effect is reversed when it binds to calcium-calmodulin complex. Hence elevated intracellular calcium concentration stimulates calcium efflux and so helps avoid cytosolic calcium overload (Katz 2001f).

Even though the sarcolemmal Ca^{2+} ATP-ase has a relatively high affinity for Ca^{2+} it plays a small (almost negligible) role in removal of Ca^{2+} from cardiomyocytes (Carafoli 1985). Its capacity for removing Ca^{2+} is less than one tenth that of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Barry 1993). Activity of the Ca^{2+} ATP-ase is reduced during ischemia, resulting in a reduction in Ca^{2+} efflux and net Ca^{2+} uptake by the cell (Chemnitius 1985).

1.2.2.2 The sodium calcium exchanger.

This antiport can carry sodium and calcium in both directions across the plasma membrane. The amount of each, and the direction of transport is determined by

the ratio between sodium and calcium concentrations on either side of the membrane.

The main driving force for calcium efflux via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is the sodium gradient: $[\text{Na}^+]_i/[\text{Na}^+]_o$. This gradient is established by the Na^+/K^+ -ATP-ase. Thus the energy source for the “uphill” transport of calcium out of the cell via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is the ATP used by the Na^+/K^+ -ATP-ase to establish the sodium gradient.

The sodium calcium exchanger is responsible for about 80% of calcium efflux from the cardiomyocyte. It is a large protein with 11 membrane spanning α helices organized in two clusters (Katz 2001c).

It mediates Ca^{2+} efflux during diastole, and in the steady state $\text{Na}^+/\text{Ca}^{2+}$ exchange extrudes all the Ca^{2+} that enters the sarcolemmal voltage gated Ca^{2+} channels, and so contributes to the decline of the $[\text{Ca}^{2+}]_i$ transient (Wier 1990). During diastole the $\text{Na}^+/\text{Ca}^{2+}$ exchanger also competes with the sarcoplasmic reticulum Ca^{2+} ATP-ase for Ca^{2+} uptake out of the cytosol, hence it helps regulate Ca^{2+} content of the sarcoplasmic reticulum (Wier 1990, Barry 1993).

Electrogenicity.

The sodium calcium exchanger is electrogenic as it transports three sodium ions in one direction and one calcium ion in the opposite direction. However, ion

fluxes through the exchanger are 1000 times slower than those through the voltage gated ion channel so the exchanger contributes no more than a few millivolts to the membrane potential (Sjodin 1980).

In the resting cell the negative intracellular potential draws sodium into the cell: Sodium follows the electrical and chemical gradient. Calcium is exchanged, so during diastole the exchanger favours calcium efflux. During systole when the cell is depolarized (the inside of the cell becomes positive) and intracellular sodium concentration is higher, three sodium ions will now leave the cell and be exchanged for a single calcium ion. Thus during systole there is a calcium influx. Calcium efflux during diastole helps to relax the heart and calcium influx during systole increases contractility. Sodium is the driver, following the electrochemical gradient, calcium is just exchanged (Katz 2001c).

During ischemia the cell membrane is depolarized and $[Na^+]_i$ is increased (Van Echteld 1991), therefore sodium will be extruded in exchange for calcium (Tani 1990, Silverman 1994). However, rapid repolarization of the cell membrane on reperfusion will favour calcium efflux in exchange for sodium.

The driving force for calcium efflux is determined by:

1. The sodium gradient across the plasma membranes. $[Na^+]_i$; $[Na^{2+}]_o$. (Barcenas-Ruiz 1987).

2. The calcium gradient across the plasma membrane: $[Ca^{2+}]_i:[Ca^{2+}]_o$ (Beukelmann 1989).
3. The membrane potential (Barceñas-Ruiz 1987, Beukelmann 1989).

The sodium-calcium exchanger and contractility (Katz 2001c).

If $[Ca^{2+}]_o:[Na^+]_o$ remains constant, the relative rates of sodium and calcium influx remains constant and there is no change in contractility.

If $[Na^+]_o$ is increased, more sodium will enter the cell causing increased calcium efflux and thus decreased intracellular calcium and decreased contractility.

If $[Ca^{2+}]_o$ is increased the ratio of $[Ca^{2+}]_o:[Na^+]_o$ will increase. Sodium efflux will increase in order to restore equilibrium and calcium influx will increase, increasing contractility.

If $[Na^+]_i$ increases there is more sodium to compete with calcium ions for binding to the intracellular side of the receptor, so calcium efflux will decrease resulting in increased intracellular calcium and increased contractility.

If $[Na^+]_i$ decreases it will have the opposite effect resulting in more calcium ion efflux and thus decreasing contractility. Cardiac glycosides make use of these principles. These drugs increase contractility by inhibiting the Na^+/K^+ -ATP-ase ,

resulting in increased intracellular sodium concentration, hence decreasing calcium efflux via the sodium calcium exchanger.

Regulation of the sodium calcium exchanger.

Although the sodium calcium exchanger is not ATP dependant, phosphorylation of the exchanger by ATP will accelerate the exchange (Blaustein 1977, DiPolo 1974).

When intracellular calcium is high, calcium binds to a high affinity site on the exchanger in the cytosol, separate from the calcium transport site and stimulates the turnover of the exchanger (this helps prevent intracellular calcium overload).

Protein kinases A and C and calcium-calmodulin kinase may also activate the exchanger.

It is possible that calcium entry via the sodium calcium exchanger inhibits calcium release from the sarcoplasmic reticulum, but this is controversial (LeBlanc and Hume 1990).

1.2.2.3 The sodium pump ($\text{Na}^+/\text{K}^+\text{ATP-ase}$).

This pump uses energy from ATP hydrolysis for the active transport of sodium and potassium across the plasma membrane. It clears the cytosol of the small

amount of sodium that enters through the voltage gated sodium channel during each action potential. The pump exchanges this sodium for the small amount of potassium which is lost during repolarization (Katz 2001c).

Both sodium efflux and potassium influx occur against the chemical gradient and so the process requires energy. The fact that the pump exchanges positively charged sodium for potassium ions, helps minimize the electrochemical work (Katz 2001c).

The sodium pump creates a large sodium gradient across the plasma membrane ($[Na^+]_o \gg [Na^+]_i$). This provides potential energy to drive the sodium calcium exchanger and the sodium hydrogen exchanger, and therefore to drive calcium and protons respectively, out of the cell, against their electrochemical gradient.

The sodium gradient also provides the driving force for the inward sodium currents that depolarize the cardiomyocyte (Katz 2001c).

Electrogenicity.

The sodium pump generates an electric current that repolarizes the cell, as three sodium ions are transported out of the cell in exchange for two potassium ions brought in. This increases the negativity of the cell interior.

During diastole the pump maintains the resting membrane potential. During systole when the myocyte is depolarized the sodium pump repolarizes the cell. During ischemia, once ATP is depleted, the sodium pump fails and the cell becomes and remains depolarized. On reperfusion, ATP is immediately available and the sodium pump rapidly repolarizes the cell membrane.

Drugs or conditions that inhibit the sodium pump tend to depolarize the cell and decrease the potassium gradient across the cell membrane. The resting membrane potential is now more positive and closer to the threshold, causing dysrhythmias (Katz 2001c).

Structure.

The sodium pump has three subunits. The largest is the α -subunit which contains the phosphorylation site for ATP and the sodium and potassium binding sites. ATP and sodium bind to separate sites in the cytosol and the potassium binds on the extracellular surface. (Glycosides compete with potassium for its binding site). The β -subunit regulates sodium and potassium binding (Katz 2001c).

Regulation.

Protein kinase A and C accelerate the pump.

Sympathetic activity increases cAMP, which activates phosphorylation and so stimulates the pump.

High ATP concentrations accelerate the reactions.

Acidosis inhibits the Na^+/K^+ ATP-ase (Piwnicka-Worms 1985, Lazdunski 1985).

1.2.2.4 The sodium hydrogen exchanger (Na^+/H^+ exchanger).

Acidosis reduces myocardial contractility because protons compete with calcium for binding sites on the contractile proteins.

Protons are generated by anaerobic glycolysis and oxidative metabolism. Intracellular pH must be maintained at 7.2, maintaining this low $[\text{H}^+]_i$ requires the following active transport mechanisms.

- Sodium-bicarbonate symport.
- Chloride bicarbonate exchange.
- Chloride hydroxyl exchange.
- Sodium-hydrogen exchange (most important).

The Na^+/H^+ exchanger uses the energy of the sodium gradient generated by the sodium pump to transport protons out of the cell. Hydrogen is exchanged for sodium in a 1:1 exchange and so is electrically neutral (Katz 2001c). Na^+/H^+ exchange therefore, regulates pH balance in myocytes (Imai 1991).

Structure (Katz 2001c).

The major Na^+/H^+ exchanger isoform in cardiac plasma membrane is a large glycoprotein with 12 membrane spanning α -helices, an N-terminal that mediates the ion exchange, and a large intracellular C-terminal region that contains regulatory sites.

Regulation (Katz 2001c).

- Intracellular acidosis activates this antiport.
- ATP and increased cytosolic calcium potentiate its action.
- When the exchanger is phosphorylated by protein kinases A and C it can mediate signals that regulate protein synthesis and apoptosis.

1.2.3 The sarcoplasmic reticulum: (SR).

Myocardial contractility is controlled by calcium fluxes across the sarcoplasmic reticulum. This is the intracellular calcium cycle. These calcium fluxes are stimulated by sympathetic activation and inhibited by acidosis and decreased cytosolic ATP levels.

Calcium uptake and release from the sarcoplasmic reticulum are both highly regulated. $[\text{Ca}^{2+}]$ in the sarcoplasmic reticulum is high, hence calcium release during excitation is a passive process which accounts for 90% of the calcium

transient in mammals (Wier 1990, Barry 1993). Calcium uptake by the sarcoplasmic reticulum, which relaxes the heart, is however energy dependant (Katz 2001d).

1.2.3.1 **Structure of the sarcoplasmic reticulum (Fig. 1.5).**

There are two important regions (Katz 2001d).

1. The **subsarcolemmal cisternae** which contain the calcium release channels. The cisternae form special junctions with the plasma membrane and t-tubules called dyads. At these dyads the plasma membrane and t-tubules contain a high concentration of L-type voltage gated calcium channels which are in close proximity to the calcium release channels on the cisternae of the sarcoplasmic reticulum. These calcium release channels are also called ryanodine receptors.
2. An extensive **sarco tubular network** that contains the Ca^{2+} -ATP-ase pump proteins responsible for calcium uptake by the sarcoplasmic reticulum.

1.2.3.2 **Calcium release channels (ryanodine receptors).**

In cardiac muscle membrane depolarization leads to opening of the L-type voltage gated calcium channels. Calcium flows into the dyad and generates a chemical signal that opens the calcium release channels in the cisternae of the

sarcoplasmic reticulum (Anderson 1989, Katz 2001f). Calcium released by the sarcoplasmic reticulum is responsible for the initial rapid increase in $[Ca^{2+}]_i$ after depolarization (Barcenas-Ruiz 1987, Beukelmann 1988).

Intracellular calcium release channels and plasma membrane L-type calcium channels are not randomly scattered across the surface of the dyad, they are grouped together in functional clusters (Sham 1995, Stern 1999).

The inositol triphosphate (IP_3)-gated calcium release channels play a major role in smooth muscle contraction. They have a small calcium conductance i.e. they pass ions very slowly. The function of the small number of IP_3 gated channels in the heart is unclear, they mediate the slow release of only a small amount of calcium and cannot play an important role in excitation contraction coupling. However IP_3 -gated channels are activated by neurohumoral mediators like α -agonists and angiotensin II and may play an important role in calcium activated protein syntheses, cell cycling and apoptosis (Katz 2001d).

Regulation of calcium release channels.

- Increased entry of calcium through the L-type plasma membrane calcium channels increases calcium release from the sarcoplasmic reticulum. This is the most important physiological regulator. (Beukelman 1988).
- The mechanism of calcium induced calcium release is widely accepted (Beukelmann 1988, Calleweart 1988, Isenberg 1988).

- The bigger the calcium store in the sarcoplasmic reticulum, the more calcium is released (longer and more frequent opening L-type calcium channels increases the sarcoplasmic reticulum calcium store) (Barry 1993).
- ATP promotes channel opening and a fall in ATP reduces calcium triggered calcium release and contractility.
- Xanthines initiate calcium release.
- Acidosis inhibits channel opening.
- Under conditions of calcium overload , $[Ca^{2+}]_i$ in cardiac cells can oscillate spontaneously.

1.2.3.3 The calcium pump of the sarcoplasmic reticulum (SERCA).

This pump couples ATP-hydrolysis to calcium transport across the sarcoplasmic reticulum membrane (ATP dependant pump: Ca^{2+} -ATP-ase), and has the dominant role in determining the decline of the $[Ca^{2+}]_i$ transient (Carafoli 1988, Inesi 1985).

This pump does not exchange calcium for a counter ion.

The movement of positively charged calcium ions across the sarcoplasmic reticulum membrane does not generate an electrical current, because movement of chloride and phosphate anions balance the movement of calcium ions (Katz 2001d).

SERCA is a smaller molecule than the plasma membrane calcium pump (Shull and Greeb 1998) and is regulated differently.

Regulation (Katz 2001d).

During systole the cytosolic calcium concentration is high and the pump operates rapidly. During diastole the low cytosolic calcium level is rate limiting.

- The most important regulator of SERCA is the level of cytosolic calcium.
- ATP has an important regulatory effect on calcium transport via the sarcoplasmic reticulum. ATP provides the energy for this active transport. This pump however requires very low (micromolar) ATP concentrations to function, but low ATP levels will slow it down, and ATP at high concentrations stimulates the pump.

Phospholamban (Katz 2001d).

This protein is one of the most important regulators of the cardiac sarcoplasmic reticulum, and it plays an important role in the increased inotropy and lusitropy due to sympathetic stimulation (Tada and Katz 1982).

Under basal conditions phospholamban inhibits SERCA.

β -adrenergic stimulation generates cAMP. A cAMP dependant protein kinase phosphorylates phospholamban. The inhibiting effect of phospholamban on SERCA is reversed. There is an increase in the rate of calcium uptake by the SERCA, and this accelerates relaxation (lusitropy). Phosphorylation of phospholamban also increases intracellular calcium stores and so increases contractility (inotropy).

When phospholamban is phosphorylated the SERCA is much more sensitive to $[Ca^{2+}]_i$. It will now compete more strongly with a plasma membrane calcium pump (which extrudes calcium from the cell) for calcium binding, and so calcium will be retained in the cell (and in the sarcoplasmic reticulum), increasing contractility.

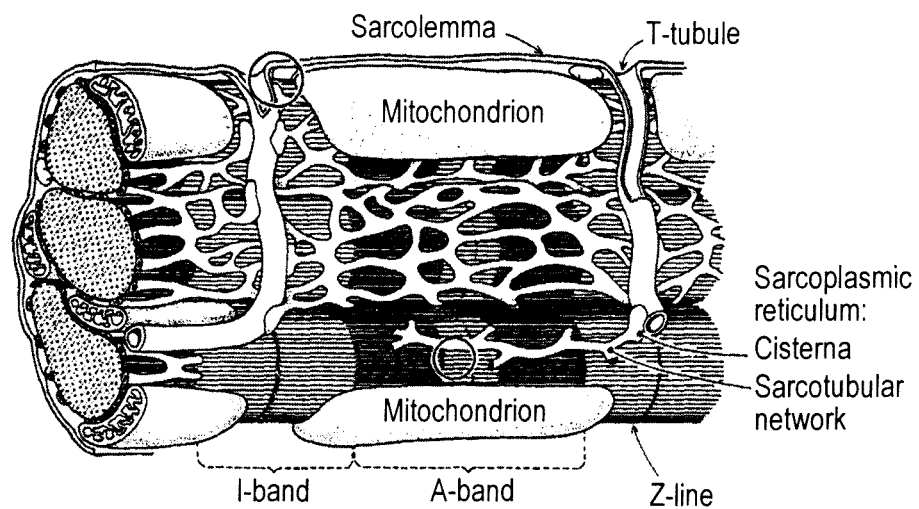


Fig 1.5

The sarcoplasmic reticulum is an intracellular membrane system surrounding the contractile proteins. It consists of the sarcotubular network and the cisternae which abut the t-tubules and the sarcolemma.

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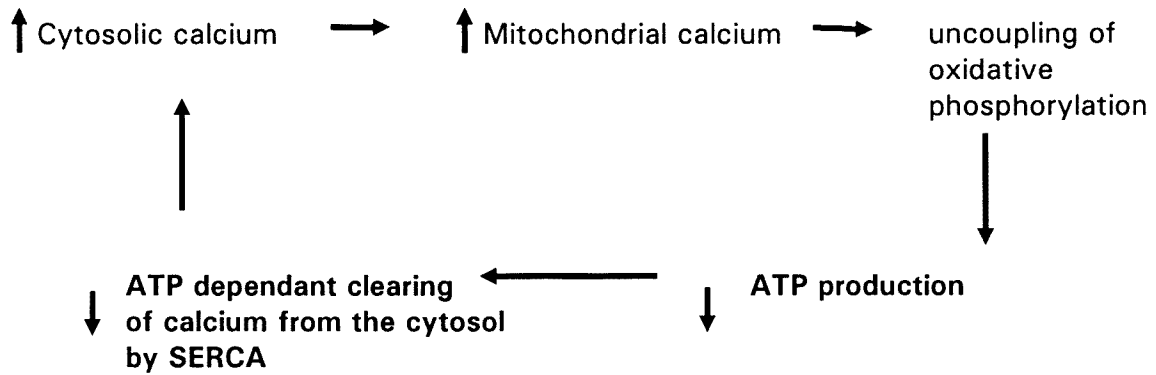
1.2.4 The mitochondrion.

In the mitochondrion the respiratory chain generates a negative intramitochondrial potential. This negative potential is a driving force for calcium uptake into the mitochondrion. However in normal cells the cytosolic calcium concentration is so low that very little calcium enters the mitochondrion.

Mitochondrial calcium uptake is too slow, and calcium affinity of the mitochondrial calcium pump too low for the mitochondrion to play an important role in relaxation of the normal heart (Katz 2001j).

Uptake of Ca^{2+} is mediated by a uniporter and is driven by mitochondrial membrane potential ($\pm 180\text{mV}$ negative to cytosol). This membrane potential is set up by outward proton pumping which is coupled to electron transport (Gunter 1990). Ca^{2+} efflux from the mitochondrion is mainly via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Gunter 1990). The maximum capacity of Ca^{2+} efflux is **one tenth** that of Ca^{2+} influx via the uniporter (Gunter 1990).

When the cytosol is calcium overloaded (ischemia and reperfusion) the mitochondria will take up calcium. This will dissipate the proton electrochemical gradient across the mitochondrial inner membrane, leading to uncoupling of oxidation from phosphorylation and impairing ATP production. A vicious cycle will be started.



A large increase in mitochondrial matrix $[Ca^{2+}]$ will activate a regulatory pathway namely the mitochondrial permeability transition pore. This megapore leads to permeabilization of the mitochondrial inner membrane to ions, including Ca^{2+} , and small solutes (Silverman 1994, Crompton 1988).

The increase in intramitochondrial $[Ca^{2+}]$ during ischemia correlates with the degree of cell injury on reperfusion (Miyata 1992). In myocytes which ultimately recover function after reperfusion, reversible rises in intramitochondrial $[Ca^{2+}]$ occur during ischemia (Silverman 1994). Blocking mitochondrial Ca^{2+} uptake during ischemia is beneficial (Gupta 1989), and inhibition of the mitochondrial megachannel by cyclosporin during ischemia, enhanced recovery of contractile function and ATP levels on reperfusion (Griffiths 1993).

1.2.5 Calcium as intracellular messenger (Katz 2001b).

Calcium ions are an important intracellular messenger. They carry signals generated at the cell surface to a variety of intracellular proteins and organelles.

Calcium is the essential final step in excitation-contraction coupling in the myocardial cell.

Kretsinger (1977) postulated how calcium became the intracellular messenger:

Phosphate anion is needed in the cell for ATP and nucleic acid (RNA and DNA) synthesis. Calcium phosphate is insoluble, so the primitive cell excluded calcium in order to concentrate phosphate anion. The result is that the calcium concentration in the extracellular fluid which is $\pm 1\text{mM}$ (like seawater), is 5000 times higher than the calcium concentration in the cytosol, which is $0.2\ \mu\text{M}$. The cell has thus effectively evolved calcium free cytosol. Now calcium entry through regulated ion channels in the plasma membrane is a useful vehicle for signaling processes in the cell. Calcium ions generate an electrical and chemical signal when they enter the cell. Calcium binding proteins have evolved to become intracellular calcium sensors.

1.2.6 Calcium cycles.

Fig. 1.6, Fig. 1.7

Calcium is available in the extracellular space and in the intracellular stores (sarcoplasmic reticulum). The large amount of calcium required for excitation-contraction coupling is provided by two calcium cycles (Katz 2001c):

The extracellular cycle where calcium enters the cell through voltage gated L-type calcium channels and efflux occurs via the plasma membrane calcium pump

(Ca²⁺-ATP-ase) and the sodium calcium exchanger. Most of the calcium that enters the cytosol via the external calcium cycle opens calcium release channels in the sarcoplasmic reticulum (calcium triggered calcium release), and only a small fraction binds to troponin C (Katz 2001d).

The intracellular cycle in which calcium moves between the stores in the sarcoplasmic reticulum and the cytosol where it binds to troponin C. The calcium fluxes out of and into the sarcoplasmic reticulum are much greater than those out of and into the extracellular fluid. The calcium that activates the contractile proteins binds to and is released from high affinity binding sites on troponin C. Mitochondria are also able to buffer high cytosolic calcium levels (Katz 2001d).

In mammalian myocardial cells calcium release from the sarcoplasmic reticulum is smaller than in skeletal muscle, and graded, and does not saturate all the available troponin C (Fabiato 1989). This allows the contractile response to be graded (Katz 2001c).

During the two cycles calcium moves among five compartments (Katz 2001d): (Fig. 1.6).

1. Extracellular space.
2. Sarcoplasmic reticulum.
3. Cytosol.

4. Contractile proteins.
5. Mitochondria.

Calcium fluxes among these compartments initiate and terminate contraction and regulate contractility (Katz 2001d).

Contractility is regulated by:

- Calcium channels (L-type voltage gated calcium channels and sarcoplasmic reticulum calcium release channels).
- Calcium exchangers.
- Calcium pumps.
- Calcium affinity of the contractile proteins.

The numbers of plasma membrane L-type calcium channels and calcium release channels on the sarcoplasmic reticulum are about the same. However calcium flux through a sarcoplasmic reticulum calcium release channel is ten times faster (750 000 ions per s) than through a L-type calcium channel (75 000 ions per s). This explains why calcium influx via the extracellular cycle is much smaller than calcium release via the sarcoplasmic reticulum.

Calcium uptake by each sarcoplasmic reticulum calcium pump is only 30 ions per second, there are however 170 times more sarcoplasmic reticulum calcium pumps than sarcoplasmic reticulum calcium release channels. The large number

of sarcoplasmic reticulum calcium pumps provide some compensation for their much slower rate, but calcium release is still twice as fast as calcium removal by the sarcoplasmic reticulum.

It is more difficult to relax than to contract, because diffusion is so much faster than active transport. Relaxation takes twice as long as contraction so the heart spend longer in diastole than in systole. Relaxation is markedly impaired in the energy starved heart (ATP is needed for the sarcoplasmic reticulum calcium pump to work). A fall in ATP concentration locks the contractile proteins in rigger bands and causes the cytosol to become flooded with calcium (Katz 2001d).

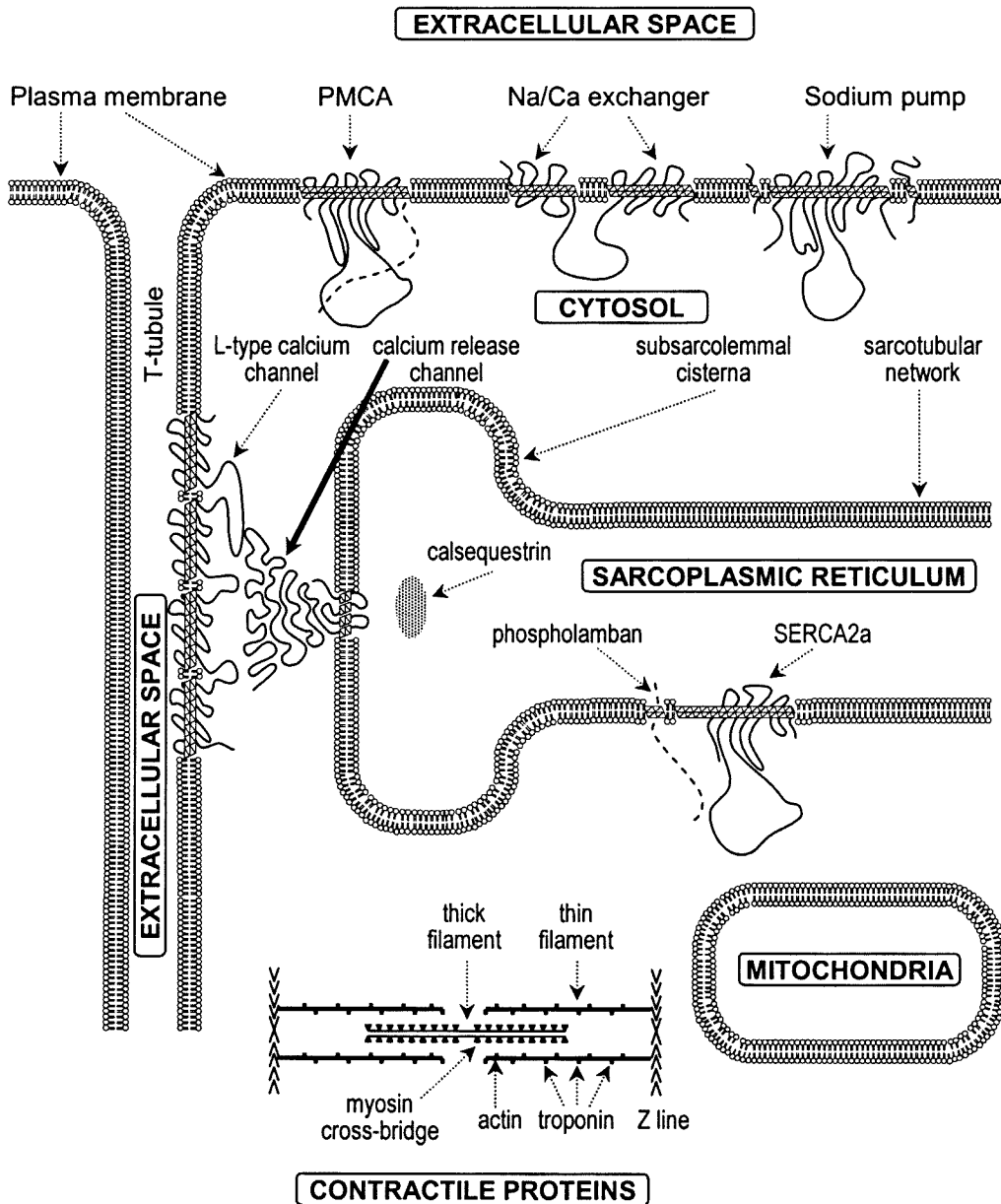


Figure 1.6

The key structures and calcium fluxes that control cardiac excitation-contraction coupling and relaxation. Calcium pools are in bold capital letters.

A Katz

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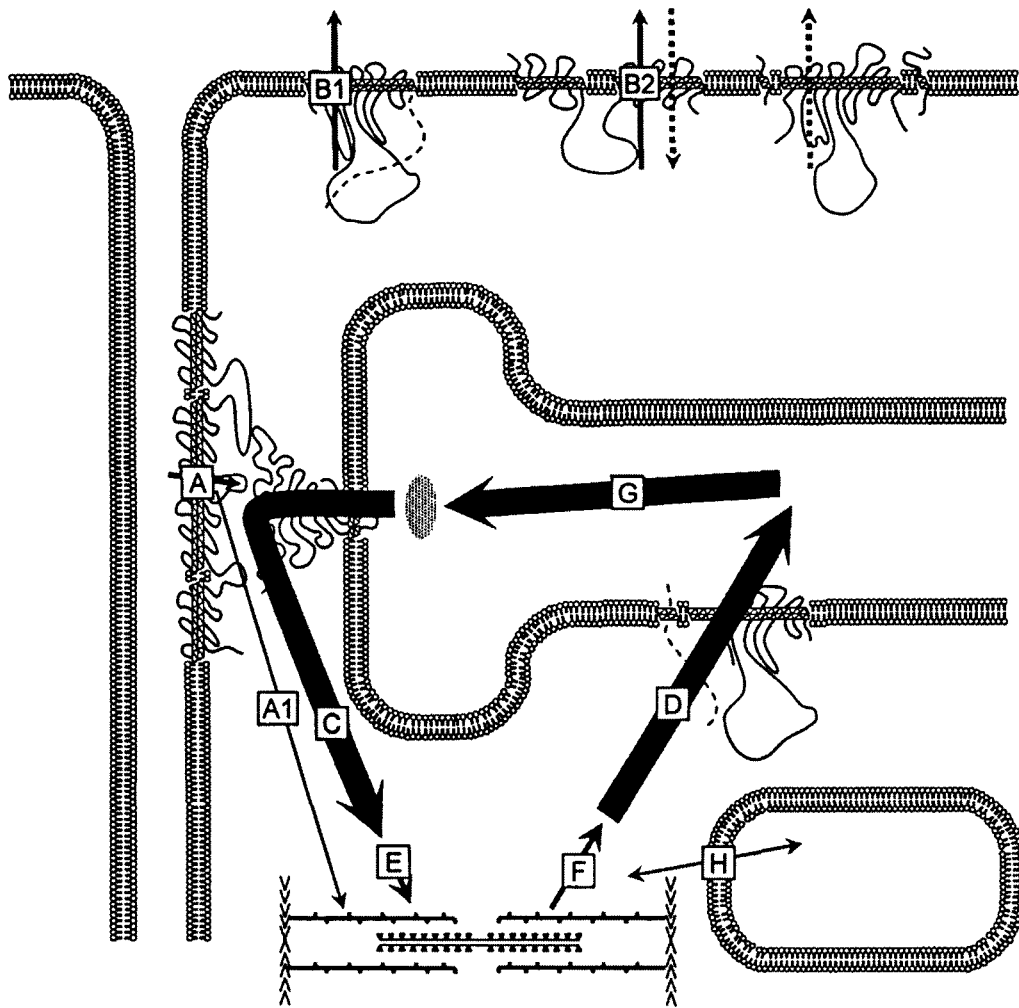


Figure 1.7

The thickness of the arrow indicates the magnitude of the calcium fluxes. Downward arrows represent passive calcium fluxes and upward arrows represent energy dependent active calcium transport. Calcium that enters the cell from the extracellular fluid via the L type calcium channels (arrow A) triggers calcium release from the SR (arrow C). Only a small amount directly activates contractile proteins (arrow A1). Calcium is actively pumped back into the extracellular fluid by plasma membrane calcium ATPase (arrow B1) and exchanged for sodium via the sodium/calcium exchanger (arrow B2). The sodium that enters the cell in exchange for calcium (dashed line) is pumped out by the sodium pump. Most cytosolic calcium is taken up by calcium ATPase pump on the SR (arrow D). Calcium diffuses within the SR from the sarcotubular network to the sarcolemmal cisternae (arrow G). Here it is stored ready for release. Calcium binding to high affinity calcium binding sites on troponin C (arrow E), activates the interaction of contractile proteins; and dissociation (arrow F) inhibits the interaction of contractile proteins. Calcium movements into and out of the mitochondria (arrow H) buffer cytosolic calcium concentration. The extracellular cycle is represented by arrow A, B1, B2; the intracellular cycle is represented by arrows C, E, F, D and G.

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1.3 Calcium channel blocking drugs and the reperfusion injury.

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1.3 Calcium channel blocking drugs and the reperfusion injury

1.3.1 Duration of ischemia.

If we are to evaluate stunning as the part of the reperfusion injury the following criteria should be met (Bolli 1992):

1. Lack of permanent damage (no infarction).
2. Eventual full mechanical recovery.
3. Fully restored coronary bloodflow.

Many experiments do not meet these criteria for stunning because of permanent ischemic induced necrosis. Hearts which are reperfused after more than 20 minutes of normothermic ischemia have extensive ultrastructural damage, remain energy depleted, and show permanent contractile dysfunction (Jennings, 1986). Total ischemia results in the rapid development of irreversible damage which is already morphologically evident after 30 minutes (Elz, 1988).

The greater the duration and severity of ischemia, the more likely there is to be serious damage to the cell membrane at the end of the ischemic period. There is then a greater chance of calcium entry by non-specific mechanisms during

subsequent reperfusion (Nayler 1988). For ischemia, without infarction, in the in vivo model, the ischemia duration should be limited to 15 to 20 minutes at normothermia. After 30 minutes of severe ischemia in various animal species it is difficult to differentiate between attenuation of stunning and tissue salvage due to calcium antagonists (Heusch 1992).

1.3.2 Porcine versus canine models.

The paucity of collateral vessels in the porcine heart eliminates the variability in collateral flow inherent in dog models (Heusch 1997). This results in a more reproducible post-ischemic dysfunction in pigs.

The canine heart has a native coronary collateral circulation, thus the more appropriate way to ascertain whether calcium channel blockers attenuate myocardial stunning in vivo, in conditions of zero flow ischemia, is to use the porcine model. Like the human heart, the porcine heart lacks preformed collateral anastomoses and has minimal xanthine oxidase activity (Dodds 1982).

1.3.3 Anesthetized open chest models vs. conscious models.

Results obtained in open chest models may be confounded by the effects of anesthesia, surgical trauma, abnormal hemodynamic conditions, excessive levels of circulating catecholamines, and other factors. Certain conclusions derived

from open chest preparations may indeed not be applicable to conscious models. (Bolli 1986, Ning 1990).

It has been shown that in the 15 minute coronary occlusion model of myocardial stunning, both the severity of post-ischemic dysfunction (Triana 1991) and the magnitude of free radical generation (Li 1993) are greatly exaggerated in the open chest as compared to conscious models.

The stunned myocardium, like the myopathic heart, is sensitive to hemodynamic variables, hence reduction in afterload, variations in preload, heart rate and regional myocardial blood flow, could increase systolic shortening independently of changes in intrinsic contractile properties (Przyklenk 1989).

1.3.4 Effects of calcium channel blocking drugs.

1.3.4.1 How do calcium channel blocking drugs attenuate the reperfusion injury?

In the in vivo model, when calcium antagonists are administered systemically (intravenously), there usually is a decrease in arterial pressure resulting in a decreased afterload and an improvement of ejection function, as well as baroreceptor mediated increased cardiac sympathetic nerve activity. However, even in studies where afterload was kept constant (Böhm 1991) or calcium antagonists were administered into coronary arteries, with minimal systemic

effects (Przyklenk 1989, Rohmann 1991), there was improved recovery of regional contractile function compared to placebo.

It has been suggested that calcium antagonists may attenuate stunning by reducing the severity of the preceding ischemia due to increased myocardial blood flow via collaterals. However, in the vast majority of studies regional myocardial blood flow was not increased by calcium antagonists during ischemia and ischemic contractile function was not improved (Lamping 1985, Gross 1987, Dunlap 1989, Böhm 1991, Przyklenk 1988, Farber 1989, Taylor 1990).

Calcium antagonists may improve myocardial function due to improved blood flow during reperfusion (Gregg phenomenon: an increase in myocardial function secondary to an increase in coronary blood flow). This is probably not the underlying mechanism for improved function associated with calcium antagonists, as improved functional recovery was also found in the absence of improved blood flow during reperfusion. (Lamping 1985, Przyklenk 1989, Taylor 1990). The Gregg phenomenon is of minimal significance in the normal myocardium, but may be operative in the reperfused myocardium (Bolli 1990).

Thus the consistent attenuation of stunning by calcium antagonists cannot be explained only by more favorable hemodynamic conditions such as reduced afterload, or increased myocardial bloodflow during ischemia or reperfusion.

Studies have suggested that an important mechanism involved in the protective effect of calcium antagonists in isolated preparations, is indirect. Due to the ability of the calcium antagonists to spare ATP during ischemia, as a consequence of their negative inotropic properties, they could induce some protection against ischemia, resulting in less stunning (Klein 1984, Reimer 1985).

Boraso (1993) showed that the protective effect of lacidipine is independent of a cardioplegic action occurring before ischemia, as oxygen consumption in their preparation was not affected by the drug.

Kirkels (1992) demonstrated that anipamil (lipophilic verapamil) protects against ischemia and reperfusion in the absence of a negative inotropic effect.

It has also been suggested that damage to the myocardial vascular endothelium is involved in myocardial injury following ischemia and reperfusion (Yao 1994). The mechanism for this endothelial injury may be platelet- or leucocyte-endothelial interaction and oxygen free radicals delivered from neutrophils or generated on re-oxygenation of ischemic tissue. Some calcium antagonists eg. benidipin can prevent this vascular dysfunction (Becker 2004, Kitakaze 2001).

The effect of calcium antagonists on the attenuation of stunning is probably due to the attenuation of cytosolic calcium overload during early reperfusion and the protection of the membrane against free radical induced damage. (Amende

1991, Janero 1989). Different types of calcium antagonists are all equally effective in improving functional recovery of the stunned myocardium (Watts 1986).

Gallopamil administered at the onset of reperfusion in the isolated guinea pig heart resulted in improved recovery of contractile function. Gallopamil has no oxygen radical scavenging effect and its protective effect is probably purely due to a reduction in calcium overload (Massoudi 1995).

Binding of a drug to a receptor within an ion channel is influenced by the state of the channel, which in turn is determined by the membrane potential. Binding of the calcium antagonists is approximately 1000 times stronger to inactivated channels than to resting channels (Sanguinetti and Kass 1984) (Bean 1984). Verapamil and diltiazem bind the calcium channel in the open and inactive state (Kanaya 1983). The block will therefore be greater if the channel is opened frequently, or if it stays in the inactive or depolarized state. Nifedipine and nisoldipine bind the calcium receptor in the calcium channel in the inactive state (when the membrane is depolarized). As smooth muscle cells of the vasculature spend a longer time in the depolarized state, this explains the vascular selectivity of these drugs (Sanguinetti 1984). Green (1985) observed that in isolated myocytes binding sites of nitrendipine a dihydropyridine calcium antagonist, reversibly increased by 115% as a function of increasing membrane depolarization.

During ischemia the sarcolemma is depolarized and the calcium channels are in the inactive state. At the onset of reperfusion there is rapid calcium cycling (Piper 2004) and more channels are opened more frequently so increasing the binding of verapamil.

Thus ischemia may modify the calcium channel in such a way that calcium entry may be facilitated and that the effect of calcium antagonists is potentiated.

1.3.4.2 Effect of calcium channel blocking drugs on apoptosis.

Mitochondria play a critical regulatory role in the signal transduction pathway leading to apoptosis (Gross 1999). Calcium antagonists attenuate mitochondrial injury resulting from ischemia and reperfusion (Boraso 1993).

Calcium antagonists may prevent the formation of the mitochondrial permeability transition pore and so inhibit the release of pro-apoptotic molecules i.e. cytochrome C from the mitochondrion. Reduced calcium overloading due to calcium antagonists may further attenuate myocardial apoptosis through prevention of dephosphorylation of pro-apoptotic proteins by calcineurin. By preventing the activation of calcineurin the calcium antagonists prevent cytochrome C release from the mitochondrion (Wang 1999). Calcium overload has also been demonstrated to activate calcium- and magnesium dependant endonuclease and so results in DNA fragmentation and cell apoptosis (Gottlieb

1999). Calcium antagonists may prevent endonuclease activation and reduce myocardial apoptosis.

Calcium overload increases the generation of free radicals, which induce apoptotic cell death (Bianchi 1997, Das 1999). Calcium antagonists significantly reduce free radical generation in ischemic/reperfused tissue, and this may contribute to their anti- apoptotic effect.

1.3.4.3 Effect of calcium antagonists on NO preservation and endothelial function.

Endothelial NO is formed from L-arginine by the enzyme NO synthase. This enzyme can be inhibited competitively by L-arginine analogs such as N-monomethyl-L-arginine (L-NMMA).

Early reperfusion leads to impaired endothelium dependant vasodilatation resulting from either reduced NO formation or rapid inactivation of NO by free radicals (Lefer 1996). Augmentation of NO levels during reperfusion preserves endothelial function, reduces neutrophil accumulation and decreases myocardial injury (Lefer 1996). Thus, NO appears to be an important factor mediating protection against the ischemia reperfusion injury.

Gourine (2001) showed that local administration of clevidipine during the last 10 minutes of severe ischemia and early reperfusion reduces infarct size and

preserves endothelial function. These effects are largely dependant on maintained local bio availability of NO.

Zhang (1999) showed that amlodipine releases NO from coronary microvessels. Kitakaze (2001) demonstrated that benidipine and nifedipine increased coronary blood flow during low flow ischemia, attenuated the severity of myocardial ischemia and increased nitrate/nitrite level in the coronary sinus. All these effects were mediated via a NO-dependant mechanism.

1.3.4.4 Effect of calcium channel blocking drugs on oxidant production in human neutrophils.

Polymorphonuclear leucocytes are a major source of toxic oxidants in vivo during ischemia and reperfusion injury and calcium ions are a key mediator in the production of oxidants by these cells (Romeo 1975, Goldstein 1975).

Feng (1996) examined freshly prepared human neutrophils suspended in a luminol media and mixed with varying amounts of diltiazem, nifedipine and verapamil. They found that calcium antagonists inhibited oxidant production by neutrophils (all three agents were effective).

No drug sensitive calcium channels have yet been demonstrated in neutrophils (Pennington 1986), but protein kinase C is located in the plasma membrane (Hirota 1985) and NADPH oxidase is associated with the plasma membrane

(Babior 1984). It is possible that calcium antagonists may inhibit NADPH oxidase directly and so block signal transmission responsible for oxidant generation (Feng 1996).

Very high concentrations of drug are required to suppress oxidant production (Feng 1996). However because these drugs are highly lipophilic the concentrations of the drug may be 1000 fold higher in the cell membrane than in the plasma (Hamman 1984). The drug may also accumulate in high concentration in neutrophil membranes and inhibit NADPH oxidase and protein kinase C.

1.3.4.5 Summary of calcium antagonist effects during ischemia and reperfusion.

Effects of calcium antagonists during ischemia.

Calcium channel blockers prevent ATP depletion during ischemia (Lange 1984). They also preserve mitochondrial oxidative phosphorylation during ischemia and on reperfusion (Nayler 1980). Therefore if the calcium antagonist is present from the onset of ischemia, ATP stores, ATP dependant processes, and ATP dependent pump function (Na^+/K^+ ATP-ase, Ca^{2+} ATP-ase) are better preserved during ischemia, and their recovery is enhanced on reperfusion.

Verapamil treatment before ischemia has been shown to prevent the reduction of Na^+/K^+ ATP-ase activity in membrane vesicles from isolated perfused ischemic hearts (Daly 1985). Preservation of Na^+/K^+ ATP pump function **attenuated** intracellular **sodium ion accumulation** during ischemia (Balshi 1985, Fiolet 1984, Pike 1988, Tani 1988). Nayler (1976) also demonstrated that verapamil **reduces sodium ion uptake** and potassium loss during ischemia. Calcium antagonists also have a direct interaction with the voltage gated sodium ion channel (Yatani 1985) and **reduce the influx of sodium ion** into myocytes (Grima 1987). Furthermore, pretreatment with calcium antagonists has consistently been shown to attenuate intracellular acidosis during ischemia (Kirkels 1992, Bernstein 1996). A lower $[\text{H}^+]_i$, means less H^+ exchange for Na^+ by the Na^+/H^+ exchanger and consequently **less intracellular accumulation of Na^+** .

Maintenance of Na^+/K^+ ATP-ase activity and inhibition of the Na^+ channel and the Na^+/H^+ exchanger all result in less intracellular sodium ion accumulation, and therefore a **reduction of Ca^{2+} influx** via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger.

Verapamil (calcium antagonist) pretreatment would therefore **reduce** the tendency of the myocyte in general, and the mitochondrion in particular, to become **overloaded** with **calcium** during ischemia (Bourdillon 1982, Nayler 1980). **Prevention of mitochondrial calcium overloading** preserves the ATP generating capacity of the mitochondrion (Nayler 1980, Robb-Nicholson 1978).

It is possible that maintenance of the enzymatic profile of the sarcolemma at or near normal levels, due to ATP preservation despite periods of ischemia, could contribute to verapamil's ability to protect against the deleterious effects of ischemia (Daly 1985).

Several investigators have demonstrated that treatment with calcium channel blockers before coronary artery occlusion will decrease calcium influx (Bean 1984, Ashraf 1984, Nayler 1980, Bourdillon 1982).

Other effects of calcium antagonists during ischemia are attenuation of alpha adrenergic vaso-constrictor effect (Serruys 1983, Nayler 1988), and antagonism of endothelin induced coronary vasoconstriction (Nayler 1990).

Possible effects of calcium antagonists on reperfusion mediated cell damage.

- By blocking calcium entry into reperfused cells, they reduce calcium induced calcium release from the sarcoplasmic reticulum, hence prevent rapid calcium cycling and calcium overload, and therefore decrease hypercontracture (Piper 2004).
- Inhibit oxygen free radical mediated peroxidation of membrane lipids (Janero 1988; Mak 1990 and 1992).
- Reduce activation of stimulated neutrophils and suppress the capacity of neutrophils to produce oxidants (Feng 1996).
- Release nitric oxide (NO) from the coronary arteries (Gourine 2001).

- Protect the vascular endothelium (Mc Donagh and Roberts 1986).
- Inhibit platelet aggregation (Zhang 1998).
- Protect the sarcolemma (Daly 1985).
- Reduce the severity of ischemia and slow the loss of adenine precursors (Przyklenk 1988, De Jong 1982).
- Reduce apoptosis by inhibiting caspase activation (Gao 2001).
- Attenuate ischemia reperfusion induced displacement of endogenous noradrenaline (Nayler 1988).

1.3.4.6 Effects of verapamil and mechanisms by which verapamil might improve contractile function in the stunned myocardium.

General effects of verapamil (Ferarri 1997)

- It has direct myocardial and electrophysiological effects – it reduces inotropism and heart rate.
- It is cardioprotective in a variety of experimental models even after ischemia.
- It does not induce reflex sympathetic activation.
- It may reduce myocardial catecholamine content.
- It is the only calcium antagonist proven to improve prognosis post myocardial infarction.

Mechanisms by which verapamil might improve contractile function in the stunned myocardium (Przyklenk 1989).

- During ischemia, and especially at reperfusion, intracellular calcium overload leads to hypercontracture and production of cytotoxic free radicals. Verapamil has a specific action as a calcium channel blocker on the myocyte and prevents calcium overload.
- Systemic vasodilatation and afterload reduction during occlusion or reperfusion. However beneficial results have been obtained using isolated heart preparations not complicated by changes in systemic pressures.
- Coronary vasodilatation and increased myocardial blood flow during reperfusion.

In summary:

Verapamil administered before ischemia will:

- Maintain calcium homeostasis during occlusion.
- Decrease mean arterial pressure during occlusion.
- Maintain calcium homeostasis at reperfusion.
- Increase regional myocardial bloodflow during reperfusion.

Verapamil administered at the time of reperfusion will:

- Maintain calcium homeostasis at reperfusion.
- Decrease mean arterial pressure during reperfusion.

- Increase regional myocardial blood flow during reperfusion.

Verapamil administered post reperfusion will:

- Decrease mean arterial pressure during reperfusion.
- Increase regional myocardial blood flow during reperfusion.

1.3.5 Timing of calcium antagonist administration.

The increase in cytosolic calcium **during** ischemia has very little to do with voltage gated calcium channels which are blocked by calcium antagonists as these channels are sensitive to both acidosis and decreased ATP, and are inactivated within 5 – 15 minutes after the onset of ischemia (Sperelakis 1988).

However during the first few moments of reperfusion calcium influx through the voltage gated calcium channels triggers massive calcium release from the sarcoplasmic reticulum and this causes contraction band necrosis. To be effective the calcium antagonist must be present in an adequate dose at the **onset** of reperfusion, which it will be if it is administered before or during ischemia, but not if administration is delayed until after the onset of reperfusion.

The increased cytosolic calcium levels due to ischemia and reperfusion usually return to normal within a few minutes after reperfusion, by which time the damage has already been done (Steenbergen 1987, Guarnieri 1989).

From the work of Du Toit and Opie (1992) it is evident that the timing of the calcium antagonist administration is very important. Early nisoldipine avoided subsequent stunning in the isolated rat heart. Late nisoldipine given to isolated rat hearts already stunned, decreased contractile function. The early protective effect is due to the inhibition of calcium entry via the voltage gated L-type calcium channels and consequently less calcium triggered calcium release from the sarcoplasmic reticulum. The degree of stunning is proportional to the calcium ion concentration in the early reperfusion medium. The higher the dose of nisoldipine, the lower the calcium ion concentration.

The early reperfusion period has been demonstrated to be the most important period for treatment with calcium antagonists. Herzog (1997) showed that low dose intracoronary diltiazem delivered exclusively during early reperfusion diminished infarct size in swine.

Ryanodine, which inhibits sarcoplasmic reticulum release of calcium, has a similar effect if present at the onset of reperfusion (Du Toit 1992). A reduction in stunning in the vivo dog heart is seen if verapamil is present at the onset of reperfusion (Przyklenk 1989). Later, following established reperfusion, cytosolic calcium concentration in the stunned myocytes is low, and calcium antagonists given this time will exacerbate the myopathy.

In most of the earlier studies treatment with calcium antagonists was started either before ischemia (where it was generally effective), or after reperfusion

was already established for at least several minutes (where it was generally ineffective).

The reason why calcium antagonists are beneficial only when given before reperfusion may be that the time required for drug binding and action is longer than the time required for calcium overload on reperfusion (Taylor 1990).

Segawa (2002) used the ultra short acting drug clevidipine ($t_{1/2}$ in pigs 0.5 minutes) administered directly into the left anterior descending coronary artery (LAD) to demonstrate the importance of timing of calcium antagonist administration. Clevidipine is effective in limiting infarct size if it is administered early during ischemia (the first 5 to 10 minutes) and especially if it is administered from 1 minute before reperfusion (for 5 minutes) but not if it is given during late ischemia. The beneficial effect was *not* due to change in afterload or increased coronary flow. This indicates that calcium influx, both during the early phase of ischemia and at the time of reperfusion, is involved in tissue damage induced by ischemia.

The increase in calcium influx into the cell occurs mainly in the very early phase of ischemia and during the first few minutes of reperfusion. The calcium current is markedly reduced or absent within 15 minutes of ischemia due to low pH and ATP levels (Sperelakis 1988). Consequently the beneficial effect of clevidipine given between 5 to 10 minutes of ischemia and just before reperfusion may be due to blockade of calcium entry through the L-channel. Administration of

clevudipine after 35 minutes of ischemia is too late to influence the calcium influx during ischemia and too early to influence calcium influx during reperfusion.

Long acting calcium antagonists administered before ischemia may remain in the jeopardized myocardium not only throughout the ischemic period, but also up to the onset of reperfusion and so exert a protective effect during both ischemia and reperfusion.

In one study intracoronary administration of nifedipine 30 minutes after reperfusion in dogs led to marked improvement in contractile function. (Przyklenk 1989). However, other studies have failed to document any protective effect following delayed administration of calcium antagonists. The interpretation of these results is uncertain:

- Nifedipine is a vascular selective calcium antagonist and would be expected to exert coronary vasodilator effects before effects on the myocardium.
- The result is not due to protection against intracellular calcium overload as calcium levels return to normal within a few minutes of reperfusion.
- The result is not due to the protection of membranes from free radicals, as free radicals responsible for stunning are the ones generated in the first few minutes of reperfusion. Also, 80 to 1000

times higher dose of nifedipine is required for a 50% attenuation of free radical induced lipid peroxidation of myocardial membranes in vitro (Janero 1989).

If calcium antagonists are given before or at the onset of ischemia they may attenuate ischemia. The severity of ischemia determines the severity of the reperfusion damage (Bolli 1990, Li 1993), so the calcium antagonists indirectly diminish the reperfusion injury.

When calcium antagonists are administered before or exactly at the time of reperfusion they probably specifically limit the entry of calcium ions via the calcium channels and so diminish pathogenic cytosolic calcium oscillations.

Post reperfusion administration of calcium antagonists in the presence of established stunning is of controversial significance (Opie 1994). Intravenously given agents could act by altering hemodynamic parameters. Where loading conditions are constant the calcium antagonist either has no effect (Ehring 1992) or a marked negative inotropic effect (Du Toit 1992).

In conclusion, it appears that there is sufficient evidence that calcium antagonists are indeed useful in attenuating myocardial stunning, but that timing of the administration is of vital importance. There seems to be a very narrow window of benefit.

1.3.6 A summary of studies where calcium antagonists were administered before or during ischemia in in vitro models.

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Nayler WB 1976	15 min total	Verapamil 0.5-1mg.t ⁻¹	Isolated Langendorff perfused rabbit hearts	Less ultrastructural damage less ATP & CP depletion	
Yamamoto F 1983	35 min 35°C total	Diltiazem 0.21 mg/l	Isolated Langendorff perfused rat hearts. Infusion of St. Thomas cardioplegic solution with/without diltiazem for 3 min before ischemia	Improved post ischemic aortic flow. Reduced post ischemic creatine kinase leakage (40%↓)	Diltiazem afforded no additional protection under hypothermic conditions (20°C 180min)
De Jong 1982	10 – 15 min subtotal	Nifedipine 3x10 ⁻⁸ M - 3x10 ⁻⁶ M	Isolated rat heart Nifedipine given 10 min before ischemia	Less stunning	Metabolic improvement during ischemia
Yamamoto 1983	30 min 37°C total	Verapamil 0.5mg/l	Isolated working rat heart 1. Verapamil infusion for 20 min before ischemia/or placebo. 2. Infusion of St Thomas solution with verapamil for 3 min before ischemia	Increased recovery of aortic flow CO and SV ↓ creatinine kinase leak	No additional protection during hypothermia. Post ischemic verapamil treatment had no benefit.
Watts 1986	33 min 37°C total	Verapamil Diltiazem Nifedipine	Isolated working rat hearts. Drugs given before ischemia	Enhanced recovery of contractile function. Less intracellular calcium overload	No protection if given on reperfusion
Ferrari 1986	60 min total	Nifedipine	Isolated rabbit heart – 10 min before ischemia	Improved mechanical function (only slight)	No protection if given on reperfusion. Long ischemic time.
Van Amsterdam etal 1990	30 min total	Gallopamil Diltiazem Bepridil	Isolated rat heart administered before ischemia	Faster and better recovery of contractility	Vasodilatory isomers were used
Nayler 1994	15 min total	Nisoldipine 10 ⁻⁸ M	Isolated rat heart administered before ischemia	Stunning eliminated	Striking benefit
Buser K 1991	30 min total	Verapamil Nifedipine	Isolated hypertrophic working rat heart. <u>3 weeks or 3 days before ischemia</u>	Improved recovery of contractile function	Recovery <u>much better</u> in rats on <u>chronic</u> verapamil treatment.
Du Toit 1992	20 min total	Nisoldipine 10 ⁻⁸ m	Isolated rat heart. 2 min before ischemia	Improved mechanical function	No stunning
Ferrari 1992	15 min total	Nisoldipine 0.3mg/kg/day	Isolated rabbit heart – chronic oral pre treatment	Stunning eliminated	
Mitchell 1992	27 min 37°C total	Nicardipine 4μg	Isolated working rat hearts. Nicardipine in cardioplegia before ischemic arrest	Significantly better recovery of aortic flow, CO, SV	No benefit during <u>hypothermia</u> 10°C
Baxter 1993	10 min	Diltiazem 18μg	Isolated hypertrophic working rat heart.	A very marked reduction in incidence and duration of VT VF	Also very effective in non hypertrophied hearts
Kirkels, 1993	30 min	Anipamil 5mg/kg bd for 5 days	Isolated rat hearts: Rats pretreated with Anipamil for 5 days	Greatly improved functional and metabolic recovery (p < 0.0005)	Protection <u>not</u> preceded by: ↓BP, ↓HR, negative inotropic effect energy sparing during ischemia. But intracellular pH higher in treated group during ischemia

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Boraso 1993	30 min total	Lacidipine 10^{-8} M	Isolated rabbit hearts infusion of drug for 2 hours ending 1 hour before ischemia	Improved functional recovery, ATP, CP stores, mitochondrial function. Reduced: tissue & mitochondrial calcium overload, NA release, oxidative stress	Not attributed to energy sparing effect or improved O_2 delivery
Grover 1993	25 min total	Pyranquinoline (1 – 10μ M) of cromakalim (K_{ATP} opener)	Isolated rat hearts. Drug given for 10 minutes before ischemia	Significant improvement in post ischemic contractile function ↓ lactate dehydrogenase release	Drug has no K_{ATP} effect only calcium antagonist. At 1μ M – no negative inotropic effect
Yao 1994	30 min total	Benidipine 1 or 10^{-6} M	Isolated Langerdroff-perfusol rat heart. Drug given from 20 min. before to onset of ischemia	Improved contractile function ↓ release: LDH and CPK preserved vasodilator response to acetylcholine	This calcium antagonist clearly prevents post ischemic vascular dysfunction. At 1 nM – no negative inotrope effect
Massoudy 1995	15 min total	Gallopamil 0.1 + 1 nM	Isolated working guinea pig heart. Drug given before ischemia	Improved contractile function less oxidative stress	As effective if given on reperfusion 0.1 nM – no negative inotrope effect
Bernstein 1996	90 min low flow 22-24% of baseline	Felodipine 2×10^{-6} M	Isolated isovolumic blood perfused rabbit hearts paced at constant HR. Drug started after 15 min of ischemia until 15 min of reperfusion	Improved ischemic diastolic dysfunction. Improved post ischemic contractile function. Improved post ischemic diastolic dysfunction. Improved post ischemic coronary blood flow	No negative inotropic effect. Less contracture
Dagenais 1997	30 min total	Diltiazem } 10^{-9} M Nifedipine } Verapamil } 10^{-6} M	Isolated rat hearts. 10 min. infusion of drug before ischemia	Improved endothelium-dependant relaxation after ischemia in all 3 drugs-dose dependant (nifedipine most potent)	Low dose no negative inotropy
Lochner 1998	45 min total	Halothane: 1.5% Nifedipine: 10^{-8} - 10^{-6} M Halothane + Nifedipine	Isolated rat heart. Controls + Halothane alone during cardioplegia. Nifedipine for 10 min before cardioplegia and for 1" 10 min of reperfusion. -Halothane + nifedipine	Both halothane and nifedipine were protective-(nifedipine better than halothane). The combination offered no added protection	Halothanes protective effect may be due to inhibition of Ca^{2+} influx via voltage gated Ca channels
Nayler 1990	10 min isch then 15 min reperfusion x 3	Nifedipine 10^{-8} M Verapamil 10^{-8} M Diltiazem 10^{-7} M Amlodipine 10^{-8} M Felodipine 10^{-8} M	Isolated rat heart. Drug for 15 min before first occlusion and kept in perfusion medium throughout. (Before, during, after ischemia)	Total recovery of contractile function with all drugs after all 3 episodes of ischemia	Not due to improved myocardial perfusion

CO – cardiac output, VT – ventricular tachycardia

SV – stroke volume, VF – ventricular fibrillation

↑ - increase, ↓ - decrease

→ - gave rise to

1.3.7 A summary of studies where calcium antagonists were administered at or after reperfusion in in vitro models.

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Yamamoto 1983	30 min 37° total	Verapamil 0.5mg/l	Isolated working rat heart. Infusion for the first 15 min of reperfusion	No improvement in functional recovery	Does improve recovery if given before ischemia
Watts 1986	33 min 37° total	Verapamil Diltiazem Nifedipine	Isolated working rat hearts. Drug given at reperfusion	No improvement in functional recovery	Does improve recovery if given before ischemia
Bolluig 1983	45 min total	Verapamil $5 \times 10^{-6}M$	Isolated non working rabbit heart at 37°C	Improved LV recovery better compliance	Very long ischemic time not true stunning
Higgins and Blackburn 1984	30 min total	Nifedipine Verapamil Diltiazem	Isolated working rat heart	Improved recovery with N. Worse recovery with V + D	Temp during ischemia not controlled
Ferrari 1986	60 min total	Nifedipine	Isolated rabbit heart	No effect	Beneficial if given before ischemia – long ischemic time
Du Toit 1992	20 min total	Nisoldipine $10^{-8}M$	Isolated working rat heart. Dose given for 2 min at onset or reperfusion	Lessened stunning	Similar protection by ryanodine or magnesium
Du Toit 1992	20 min total	Nisoldipine $10^{-8}M$	Isolated working rat heart. Dose given <u>20 min after reperfusion</u>	Depressed cardiac output	
Massoudy 1995	15 min total	Gallopamil 0.1nM	Isolated working guinea pig heart. Dose <u>on reperfusion</u> for 5 min	Improved contractile function less oxidative stress	As effective as when given before ischemia, more effective in reducing oxidative stress if given on reperfusion

1.3.8 Summary of studies where calcium antagonists were administered before/during ischemia *and* at/after reperfusion in in vitro models.

Yamamoto (1983) studied the effect of verapamil in the isolated perfused working rat heart:

The addition of verapamil, 0.5mg/l to St. Thomas solution before normothermic ischemic arrest (30 minutes at 37 °C), led to an enhancement of the protection by St. Thomas solution. Under hypothermic conditions (150 minutes at 20°C) verapamil was unable to confer any additional protection. When verapamil was used instead of St. Thomas solution before normothermic ischemic arrest, it was effective in improving post ischemic function and reducing enzyme leakage. It was however not as effective as St. Thomas solution alone or as the combination of the two. Verapamil alone before hypothermic ischemic arrest afforded no protection. Addition of verapamil to the perfusion fluid for 20 minutes prior to ischemia had some beneficial effects. Infusing verapamil during the first 15 minutes of reperfusion did not improve recovery.

Watts (1986) studied Isolated working rat hearts, made ischemic for 27 to 33 minutes and thereafter reperfused:

There was no enhancement of contractile function when verapamil, diltiazem or nifedipine were given to hearts during the initial phase of reperfusion. However when these drugs were given to hearts before ischemia the recovery on reperfusion was significantly greater than hearts receiving no drug. There were no major differences between the three drugs. The protection was associated

with higher energy stores and reduced intracellular calcium overload following reperfusion.

Massoudy (1995) studied isolated working guinea pig hearts made ischemic for 15 minutes and reperfused for 15 minutes. Gallopamil was given either before ischemia (0.1nM and 1 nM) or on reperfusion (0.1nM) for the first 5 minutes of reperfusion. Short term post-ischemic application of gallopamil was as effective at restoring cardiac pump function as pre-ischemic application. Pre-ischemic application had no negative inotropic effects (0.1nM) contributing to its protection. If administered on reperfusion there was an improved reduction in oxidative stress.

1.3.9 A summary of studies of systemic administration of calcium antagonists in in vivo rodent models.

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Kinoshita 1989	5 min + LAD occlusion	Diltiazem: 0.5 + 2mg/kg Verapamil: 0.5 + 5mg/kg Nifedipine: 5 + 50µg/kg	<u>Conscious rats</u> Drugs administered 10 min before ischemia (12 in each group) Control group (15)	Immediately on reperfusion: Control group: 100% VT – 87% VF - death. Diltiazem (0.5mg/kg): 42% VF (2mg/kg): 35% VF Verapamil (0.5mg/kg): 25% VF (5mg/kg): 0%VF Nifedipine (5µg/kg): 25% VF (50µg/kg): 8% VF	Aim was to evaluate reperfusion arrhythmias. Verapamil group ↓↓ HR likely due to effect on slow calcium channel
Obata 1997	15 min LAD occlusion	Diltiazem IVI 100µg/kg/min	Anesthetized rats Drugs started before ischemia	Decrease OH • generation in reperfused segment	
Gao 2001	45 min occlusion rep 240 min	Benidipine IVI 10µ/kg 3µg/kg	Anesthetized rabbits Drug administered 10 min before ischemia	Decreased myocardial apoptosis/necrosis Reduced infarct size. Improved functional recovery (10µg/kg)	<u>Infarction</u> model. Both doses effective lower dose no hemodynamic changes

HR – heart rate

1.3.10 A summary of studies of systemic administration of calcium antagonists in in vivo canine models.

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Brooks (1980)	10 min LAD occlusion	Verapamil IVI 0.1mg/kg bolus 0.01mg/kg/min	Anesthetized dogs Verapamil infusion started 15 min before ischemia and continued throughout ischemia and reperfusion	During ischemia and reperfusion: reduced incidence of VF. Raise vulnerable period threshold for VF	Verapamil has no effect on VF threshold in normal hearts, only in ischemic hearts. ? due to antagonism of enhanced adrenergic impulse
Lamping (1985)	15 min LAD occlusion	Nicorandil IVI 100 µg/kg → 25µg/kg/min Nifedipine IVI 3µg/kg → 1µg/kg/min	Anesthetized dogs. Drug/vehicle started 10 min before and continued throughout ischemia	Significant improvement in contractile recovery in both treatment groups. No change in myocardial bloodflow during ischemia or reperfusion	<ul style="list-style-type: none"> Significant decreases in MAP during ischemia. Recovery not related to myocardial bloodflow. May be due to ↓ O₂ demands during ischemia
Wartier (1988)	10 min LAD occlusion	Nitrendipine IVI 3µg/kg/min	Conscious dogs Drug/vehicle started 30 min before ischemia, continued 30min into reperfusion	Significantly greater recovery of contractile function at 6 h of reperfusion	Nifedipine – reduced MAP increased HR and coronary blood flow
Przyklenk (1988)	15 min LAD occlusion	Verapamil IVI 0.2mg/kg bolus→0.6mg/kg/h in saline @ 1ml/min	Anesthetized dogs 1. Verapamil 30 min before ischemia throughout 15 min ischemia + 180 min reperfusion. 2. Verapamil started immediately before reperfusion. 3. Verapamil started 30 min after reperfusion	Verapamil administered at or after reperfusion significantly enhanced the function of the stunned myocardium. Verapamil administered before ischemia prevented stunning	Verapamil administered before ischemia → ↓↓ MAP not so in other groups. Verapamil no negative inotropic effect during ischemia
Lorie (1989)	90 min LAD occlusion	KT-362 IVI 300µg/kg/min x 20 min 150µg/kg/min x 80 min	Anesthetized dogs Drug /vehicle started 10 min before ischemia continued throughout ischemia	Significant improvement in systolic function. Marked reduction in infarct size	Infarction model. ↓ MAP early reperfusion
Taylor (1990)	15 min LAD occlusion. 24h reperfusion	Diltiazem 15µg/kg/min	Conscious unselected dogs. Diltiazem started 30 min before occlusion cont 3 h into reperfusion. 3 days later repeated with saline	Significant improvement of contractile function not due to decrease in MAP. Segments with most severe ischemic dysfunction benefit most	↓ MAP during diltiazem Rx no difference in regional myocardial bloodflow
Ehring (1992)	15 min circumflex occlusion	Nisoldipine 5µg/kg IVI	Anesthetized dogs 4 groups: placebo; drug before occlusion; drug at 10 min occlusion, drug at 4 min reperfusion	Significant improvement only when drug is given before ischemia no benefit during ischemia or after reperfusion	MAP kept constant (balloon) HR constant. No change in regional myocardial blood flow during ischemia or reperfusion
Hammerman (1997)	LAD occlusion 2h ischemia → 1 h reperfusion→ 1 h ischemia → 2 h reperfusion	IVI nisoldipine 6µg/kg/min	Anesthetized dogs Drug/saline started 30 min after ischemia and continued throughout	Significant reduction in infarct size in nisoldipine group	Infarction model BP + HR similar
Przyklenk (1989)	LAD occlusion 15 min reperfusion 4.5 h	IVI: nifedipine 0.5mg/kg @1ml/min for 30 min	Anesthetized dogs Drug/saline started 30 min after reperfusion continued for 30 min	Significant improvement in systolic contractile function	Sustained reduction in MAP, coronary artery bloodflow doubled

MAP – mean arterial pressure

1.3.11 Summary of studies of systemic administration of calcium antagonists in in vivo porcine models.

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Park (1996)	10 x 2 min occlusion separated by 2 min reperfusion LAD	Nisoldipine IV 0.5µg/kg/min	Conscious pigs. Drug/vehicle from 15 min before first ischemia to 30 min after last reperfusion	Significant improvement of contractile function after 10 th occlusion at 5 min 15,30 min 1h, 3h, 4h, 5h reperfusion	Systemic hemodynamics Coronary bloodflow-constant Collateral bloodflow – nil ∴ Direct cardio protection NB preconditioning may play a role
Shimizu (1998)	45 min LAD occlusion 240 min reperfusion	IV -felodipine: 3nmol/kg/min -SNP: 30 nmol/kg/min -vehicle:	Anesthetized pigs Felodipine or SNP started 3 hours before ischemia in dose to decrease MAP by 30% (controls receive vehicle infusion). Infusion continued through 240 min reperfusion	Significant reduction in infarct size in the felodipine group, but not in the SNP group despite similar reduction in afterload	<ul style="list-style-type: none"> • <u>Infarction</u> model • Beneficial effect not related to decreased afterload. • Felodipine has no negative inotropic effects
Verdouw (1983)	30 min LAD partial occlusion (25-30% of N)	IV Nifedipine 1µg/kg/min (total: 15µg/kg)	Anesthetized pigs Drug started 10 min before reperfusion continued for 15 min	Significant reduction in dysrhythmias Significant improvement in contractile function	15% ↓ systemic vascular resistance significant increase in blood flow to ischemic segment

1.3.12 Systemic versus intracoronary administration of calcium channel blocking drugs.

Systemic administration of calcium channel blocking agents is associated with potent hemodynamic and coronary vasodilator effects. The stunned myocardium is probably sensitive to both reductions in afterload and increases in regional myocardial blood flow (Stahl 1986).

Direct intracoronary administration of calcium channel blocking agents allows the use of small doses that may still achieve the same potential effect on the myocardium, without altering mean arterial pressure or pulse rate.

Intracoronary infusion of calcium antagonists results in a blood concentration in the coronary artery 2.5 to 5 times higher than the usual therapeutic plasma level (Klein 1989).

1.3.13 Summary of studies of intracoronary administration of calcium antagonists in canine models.

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Lo (1985)	3h occlusion of LAD 3 hr reperfusion	Verapamil 0.01mg/kg/min <u>intracoronary</u>	Anesthetized dogs A. Drug/saline started 90 min after occlusion continued for 1 h into reperfusion. B. Drug/saline started just before reperfusion continued for 3h	A: Substantial enhancement of myocardial salvage. B: No enhancement of myocardial salvage	Infarction model (3h ischemia) Was an adequate dose present at time of reperfusion in B group. (Long ischemia time - cells irreversibly injured)
Przyklenk (1989)	15 min LAD occlusion	Nifedipine intracoronary $1.11 - 2.78 \times 10^{-4}$ mg/kg/min x 2 h	Anesthetized dogs Infusion started 30 min after reperfusion and continued for 2h	Significant improvement in regional contractile function 5 min after infusion: 80 – 90% of preocclusion values	No change in MAP No change in regional myocardial bloodflow. The <u>only</u> study that clearly shows nifedipine also attenuates contractile function (without changing hemodynamics)

MAP – mean arterial pressure

1.3.14 A summary of studies of intracoronary administration of calcium antagonists in porcine models.

Author	Ischemic time & type	Drug & dose	Preparation	Result	Comment
Verdouw (1983)	Total LAD 10 min occlusion 20 min reperfusion repeated twice	<u>Nifedipine</u> intracoronary A: 0.05µg/kg/min (11 min) B: bolus 2µg/kg then 0.05µg/kg/min (11 min)	Anesthetized pig A or B started 1 min before occlusion Continued through ischemia stopped on reperfusion	Group A no protection Group B: less VF All control animals died – cant compare control recovery. But recovery in both groups better than controls from other studies	Nifedipine increased LAD flow before occlusion. No difference in systemic hemodynamics
Klein (1989)	Total LAD occlusion 45 min (reperfusion 3 days)	<ul style="list-style-type: none"> Diltiazem: 0.25mg/min→0.125mg/ min Nefidipine: 0.05mg/min→0.025mg/ min. Verapamil: 0.125mg/min-0.062. Saline: 	Anesthetized pigs. Intracoronary infusion started 1 min before reperfusion continued for 45 min (16 min at high dose 30 mm at half dose)	Infarct size reduction most in diltiazem group less in verapamil group and no reduction in nifedipine group	No difference in systemic hemodynamics <u>Infarction</u> model
Haton N (1993)	Total LAD occlusion 45 min reperfusion 24h	Felodipine 7nmol/kg	Anesthetized pigs infusion in great cardiac vein. Started 5 min before reperfusion continued for 30 min	Significant improvement in contractile function from 30 min reperfusion onwards Significant reduction in infarct size	Infarction model Stable systemic hemodynamics
Segawa (2000)	Total LAD occlusion 45 min reperfusion 4h	Clevidipine 0.3nmol/kg/min	Anesthetized pigs infusion in coronary venous sinus started 10 min before reperfusion continued for 30 min	Significant reduction in infarct size	Infarction model Stable systemic hemodynamics Drug short t½ ∴ effect due to local mechanism in ischemic tissue
Gourine (2001)	Total LAD occlusion 45 min reperfusion 4 h	Clevidipine 0.3nmol/kg/min	Anesthetized pigs – intracoronary infusion started 10 min before reperfusion continued for 15 min	Significant reduction in infarct size	Infarction model Stable systemic hemodynamics No differences in LAD blood flow. Protection possibly due to NO release and preservation of coronary endothelial function
Segawa (2002)	Total LAD occlusion 45 min reperfusion 4h	Clevidipine 0.3nmol/kg/min for 5 min t½ clevidipine 0.5min in pigs	Anesthetized pigs- intracoronary infusion A started 5 min after occlusion. B started 10 min before reperfusion. C started 1 min before reperfusion continued for 5 min	Significant reduction in infarct size in group A + C but not group B	Infarction model. Stable systemic hemodynamic and coronary blood flow. B too late for ischemia protection. Too early for reperfusion protection(t½ too short drug not present at reperfusion
Herzog (1997)	Total LAD occlusion 50 min reperfusion 3h	Diltiazem 2.5mg 5.6µg/kg/min for 12 min	Anesthetized pigs – intracoronary infusion. Drug started on reperfusion continued for 12 min	Significant reduction in infarct size	Infarction model Stable systemic hemodynamics

1.3.15 Attenuation of the reperfusion injury by calcium channel blocking drugs in the clinical setting.

There has been some disparity between experimental results and clinical applications (Moukarbel 2004).

Possible reasons for this are that in the animal models:

- Coronary arteries are normal.
- Coronary artery occlusion is brief.
- Therapy can be delivered on time directly into the affected area.
- High drug concentrations can be used in animal models (not safe in the clinical situation).

In the clinical setting:

- Often the drugs are not given when they are most likely to show benefit i.e. during the first 10 minutes of reperfusion.
- Large doses were used over a prolonged period of time, allowing negative effects to become apparent.

Calcium channel blockers have a narrow therapeutic window in this setting.

This window is limited by time and dose (Herzog 1997).

Nevertheless, some clinical trials have shown a reduction in reperfusion injury:

- Fewer reperfusion arrhythmias.
- Improved ejection fraction.
- Reduced mortality.

(Mourkabel 2002)

Nicolau (1996) showed that oral diltiazem given at the time of intravenous streptokinase therapy improved left ventricular function compared to placebo (after infarction).

Theroux (1998) demonstrated in 59 patients that intravenous diltiazem given just before tissue plasminogen activator for thrombolysis significantly decreased cardiac events from 41% to 13% (cardiac events: death, myocardial infarction, recurrent ischemia).

Pizzetti (2001) reported that intravenous diltiazem administered before coronary reperfusion in patients with acute myocardial infarction led to a reduction in infarct size and improved recovery of regional function.

Marangelli (2000), in the verapamil acute myocardial infarction (VAMI) trial, showed that intravenous verapamil administered before thrombolytic therapy in patients with acute anterior myocardial infarction was beneficial. The treatment group had smaller left ventricular volumes and a lower prevalence of heart failure symptoms at 3 months compared to placebo group.

The following two clinical studies showed:

- Myocardial stunning does exist in the clinical setting.
- Stunning exists in the setting of acute subendocardial myocardial infarction.
- Calcium overload plays a central role in the pathogenesis of stunning.
- Calcium antagonists are effective in attenuating stunning.

Sheiban (1993) studied 25 patients aged between 40 and 69 years with exercise induced angina and single vessel disease. All patients had at least 80% occlusion of LAD without collaterals. All patients underwent routine PTCA and all patients had normal left ventricular function. Twelve patients received nitrates (80 to 120 mg/day) and 13 patients received nisoldipine (10 – 20 mg/day) before PTCA and the same drug was continued after PTCA. Immediately before the first balloon inflation an additional 300 μ g of nitrates or 0.05 mg of nisoldipine was injected into the LAD via the balloon catheter. The inflation time was 5.5 minutes. The following statistically significant results were recorded: During ischemia, ST segment shifts were greater in the nitrate group and 8 patients in the nitrate group and 4 in the nisoldipine group experienced angina. During ischemia all patients had anterior wall akinesia. Immediately after reperfusion, 10 patients in the nisoldipine group had normal motion compared to none in the nitrate group. Fifteen minutes after reperfusion 10 patients in the nitrate group and 1 patient in the nisoldipine group had anterior wall akinesis. On day one after PTCA all the patients in the nisoldipine

had normal wall function but 8 patients in the nitrate group still had anterior wall hypokinesis. The nitrate group achieved total recovery on day 3 after the PTCA.

Immediately after reperfusion ejection fraction (EF) was normal in the nisoldipine group and still significantly decreased in the nitrate group. Fifteen minutes and 1 day after reperfusion EF was still significantly decreased in the nitrate group. Total recovery in the nitrate group was again only achieved on day 3.

During ischemia both groups had significant diastolic dysfunction. The diastolic function in the nisoldipine group normalized immediately on reperfusion, whereas the nitrate group had significant diastolic dysfunction until day 3 post PTCA.

Sheiban (1996) studied 36 patients with acute anterior myocardial infarction with single vessel LAD disease who underwent successful early PTCA. The patients were randomized to either oral nitrates plus enalapril or intracoronary nisoldipine through a guiding catheter just before PTCA, followed by oral nisoldipine plus enalapril for 6 months. The nisoldipine group again had faster recovery of systolic and diastolic left ventricular function. Exercise capacity was also significantly better at 30 days in the nisoldipine group.

Other investigators reported the following:

Werner (1988) administered 1 mg of verapamil into the post-stenotic vessel area before the second inflation during PTCA of the LAD. This resulted in a delay in anginal pain and ST-segment changes compared to the control group.

Chouairi (1995) showed a similar result in 20 patients undergoing PTCA of the LAD who were randomized to receive either bepridil or placebo before the second inflation.

Serruys (1983) injected 0.2mg of nifedipine into the coronary artery before PTCA in 11 patients, this resulted in decreased myocardial lactate production in all patients.

Kern (1990) studied the effects of intravenous diltiazem and sublingual nifedipine on myocardial ischemia in 23 patients for single vessel PTCA. They reported a small attenuation of myocardial ischemia with both agents.

Feldman (1987) intravenously administered nicardipine during PTCA and noted an increase in heart rate, a decrease in mean arterial pressure, an increase in great cardiac vein flow and a decrease in electrical indices of ischemia.

Hanet (1987) administered 0.2mg nicardipine into the coronary artery before PTCA in 12 patients. They reported a decrease in the clinical and electrical indices of myocardial ischemia and a reduction in hypoxanthine and lactate release.

Babbit (1988) and Pomerantz (1991) showed that in patients undergoing PTCA for unstable angina with coronary spasms refractory to intracoronary

nitroglycerine, intracoronary verapamil resulted in complete relief of spasms with restoration of brisk anterograde flow in all patients.

Sheiban (1992) studied 30 patients with 80% LAD stenosis and exercise induced angina (normal ventricular function), undergoing PTCA. Patients received either nisoldipine, nifedipine or nitrates for 7 days before PTCA and an additional intracoronary dose at the first balloon inflation. They showed that nisoldipine and nifedipine provided protection against the reperfusion injury (stunning). Nitrates have no significant effects. Nisoldipine seems to be more effective in preventing stunning than nifedipine.

Based on these studies, there seems to be consistent evidence of a protective effect of the calcium antagonists in the clinical setting of ischemia and reperfusion.

1.3.16 Cardiac protection by calcium channel blocking drugs in the peri-operative period

Peri-operative myocardial protection has 3 components (Preusse 1992):

1. Pre ischemic phase.
2. Ischemic phase (cardioplegic arrest).
3. Post ischemic phase (reperfusion).

The pre ischemic and ischemic phase

Calcium antagonists are effective in the treatment of chronic stable angina and hypertension and form part of the treatment in the majority of patients presenting for cardiac surgery. All calcium antagonists reduce the energy turnover of the heart (Smith 1976, Watts 1986, Nayler 1976). This leads to an improved maintenance of energy-rich phosphates which is an important prerequisite for optimal myocardial protection.

Krukenkamp (1986) reported that diltiazem and other calcium antagonists lose their protective effect at temperature below 25°C to 27°C. Several authors have been unable to prove a beneficial diltiazem effect under hypothermic conditions (Hearse 1983, Piper 1985, Yamamoto 1983). Tschirkov (1992) demonstrated that diltiazem during open heart surgery was effective only when it was administered intravenously under normothermic conditions before starting cardiopulmonary bypass (CPB) and after rewarming. It was not effective when added to cold cardioplegic solution.

The post ischemic (reperfusion) phase

Calcium antagonists may be beneficial in the first few minutes of reperfusion. However, if an adequate dose of the drug is administered systemically immediately before reperfusion, the duration of action of the drug may be much

longer than the first few minutes of reperfusion when the myocytes are calcium overloaded and the drug has a beneficial effect. After the first few minutes of reperfusion the detrimental negative inotropic and chronotropic effects of the drug persist, and this may be undesirable in the post bypass period.

Tschirkov et al. (1992) reported the following beneficial results if diltiazem was given orally 60mg 3 times per day for 5 to 7 days preoperatively plus intravenously 10mg before starting CPB plus 5 to 10mg 10 minutes before reperfusion (removing the aorta cross clamp) in patient with left ventricular hypertrophy presenting for aortic valve surgery:

- An earlier onset of cardiac arrest, which can be explained by the vasodilatory effects of diltiazem on the coronary arteries when it is given intravenously just before CPB. Rapid delivery of cardioplegia to all myocardial segments is facilitated.
- Significantly better recovery of left ventricular function and systemic hemodynamics in the first 48 hours after surgery.
- Significantly better preservation of myocardial tissue with less contraction band necrosis.

They concluded that diltiazem acts directly on the myocardium, inhibiting the inappropriate calcium flux during ischemia and reperfusion and so limiting free radical production. The decreased afterload in the post ischemic period also contributes to a more rapid recovery.

Larach (1982) reported a beneficial effect of preoperative diltiazem in coronary artery surgery, largely due to decreased pulmonary and systemic vascular resistance.

Usui et al. (1999) showed that preoperative nisoldipine treatment prevented myocardial damage and reperfusion injury. Their treatment group was given nisoldipine 10mg/day for weeks before undergoing coronary artery bypass grafting. Their results were as follows:

- Myocardial blood flow was significantly higher in the nisoldipine group after cardiopulmonary bypass.
- Serum interleukin-6 levels were significantly lower in the nisoldipine group one hour after reperfusion.
- Serum lactate dehydrogenase levels were significantly lower immediately after surgery.
- The nisoldipine group showed a better left ventricular stroke work index six hours after surgery.
- The inotrope usage was significantly lower in the nisoldipine group.

In summary, stunning is a cardiomyocyte phenomenon due to underlying calcium overload of the cardiomyocytes (Heusch 1997). Calcium antagonists attenuate stunning when administered just before or at early reperfusion but not at late reperfusion (Opie 1991), as the increased calcium levels are normalized within minutes after the onset of reperfusion (Amende 1992, Carrozza 1992, Marban 1987).

1.3.17 Possible detrimental effects of calcium antagonists during ischemia.

In the clinical setting calcium antagonists may reduce myocardial blood flow by the following mechanisms:

- The coronary arteries distal to a severe (critical) stenosis are maximally vasodilated so any reduction in perfusion pressure will decrease blood flow to the post stenotic area (Canty 1988).
- Decreased arterial pressure activates the baroreflex thus leading to tachycardia. Diastolic time is shortened so decreasing blood flow to the already maximally dilated post stenotic area. To compensate for the tachycardia (shorter diastolic perfusion time), non-stenotic normal vessels will dilate, shunting blood away from the ischemic area (Heusch 1983).
- Transmural steal: In a post stenotic myocardial region auto regulation is often still present in the subepicardium but the subendocardial vessels are already maximally vasodilated. During tachycardia or pharmacological treatment the subepicardial vessels can dilate and shunt blood away from the subendocardium resulting in ischemia (Gallagher 1980).
- Collateral steal: Vasodilatation in a non-ischemic region will cause a redistribution of blood away from the maximally dilated ischemic region. (Becker 1978).

- Post stenotic dilation decreases post stenotic perfusion pressure and so reduces blood flow within the ischemic region.

In the presence of calcium antagonists, as long as arterial blood pressure is maintained, there is no redistribution of blood flow at the expense of the subendocardial layers in the ischemic area. Calcium antagonists do not aggravate ischemia in this scenario. (Ehring 1997).

Alpha adrenergic coronary vasoconstriction contributes to the initiation and aggravation of myocardial ischemia during sympathetic activation or exercise (Hisch 1990) by narrowing epicardial dynamic stenoses.

Nifedipine in anesthetized dogs prevented α_1 -adrenergic mediated increase in epicardial coronary resistance as well as the increase in end diastolic resistance mediated by α_1 - and α_2 -adrenergic receptors during sympathetic stimulation (Hisch 1984). Intracoronary and intravenous nifedipine administration during exercise (sympathetic stimulation) prevents α_2 -adrenergic receptor mediated increase in coronary resistance distal to severe stenosis as well as contractile dysfunction and lactate production.

In dogs, during exercise, the dihydropyridine calcium antagonists improve blood flow and function in the ischemic myocardium. The mechanism is probably the recruitment of dilator reserve by a reduction in coronary vasomotor tone (Ehring 1997).

Calium antagonists may interfere with ischemic preconditioning

A transient exposure to exogenous calcium has been shown to mimic ischemic preconditioning, hence a blockade of calcium channels may interfere with this reduction of infarct size (Node 1994).

However, in pigs, nisoldipine did not influence the reduction of infarct size by ischemic preconditioning. Endogenous protective mechanisms of ischemic preconditioning appear not to be affected by nisoldipine (Ehring 1997).

1.4 Hypothesis and proposed clinical application.

Based on the preceeding information it is hypothesized that:

Verapamil, when administered in an adequate dose, directly into the LAD coronary artery supplying the ischemic segment, at normothermia, would attenuate the reperfusion injury i.e. stunning and arrhythmias.

Verapamil would be effective either when administered from the onset of ischemia (for 8 minutes) or directly before and during early reperfusion (but with enough time for drug binding and effect before reperfusion).

If given from the onset of ischemia verapamil has the following effects:

- Preservation of ATP stores during ischemia.
- Preservation of membrane ATP-ase during ischemia.
- Attenuation of intracellular Na^+ accumulation during ischemia.
- Attenuation of intracellular and mitochondrial Ca^{2+} overload during ischemia.
- Antagonism of the L-type voltage gated calcium channels and attenuation of calcium induced calcium release from the sarcoplasmic reticulum during reperfusion.

If given just before and during early reperfusion verapamil would:

- Block L-type voltage gated calcium channels on the sarcolemma.
- Attenuate calcium induced calcium release from the calcium overloaded sarcoplasmic reticulum on reperfusion.
- Reduce calcium cycling by the sarcoplasmic reticulum during early reperfusion.
- Prevent/attenuate calcium induced hypercontracture and arrhythmias.

The clinical application of this hypothesis is in protecting the reperfused heart during cardiac surgery, where the application of this method (intracoronary administration of verapamil) is a distinct possibility.

2. Methods

- 2.1 General methods.**
- 2.2 Measurements.**
- 2.3 Calculations.**
- 2.4 Statistical analysis.**

2.1 General methods.

This study was conducted with the permission of the Ethics Committee of the University of Stellenbosch Medical School (letter dated 4/3/2002) and animals were cared for in accordance with institutional and international guidelines.

Pigs of both sexes (26 – 32kg) were premedicated with intramuscular ketamine (5 mg.kg^{-1}). Intravenous access was established via an ear vein and the animals received $25 \text{ mL.kg}^{-1}.\text{h}^{-1}$ normal saline for the duration of the procedure. Anesthesia was induced with pentobarbital (8 mg.kg^{-1} ; Eutha-naze, Centaur, SA) and thiopental 2 mg.kg^{-1} and fentanyl ($16 \mu\text{g.kg}^{-1}$, Tanyl: Intramed, SA). With loss of eyelid reflex, a tracheotomy was performed, the trachea was intubated and the animals ventilated (Julian, Dräger, Germany) with 50% oxygen and nitrogen. The tidal volume was set at 12 mL.kg^{-1} and the rate adjusted to maintain the end-expired PCO_2 at 35 mmHg. Anesthesia was maintained with a constant infusion of pentobarbital ($3 \text{ mg.kg}^{-1}.\text{h}^{-1}$) and fentanyl ($14 \mu\text{g.kg}^{-1}.\text{h}^{-1}$). Muscle relaxation was achieved with pancuronium bromide (Organon, Sanofi-Synthelabo) initially administered as a bolus of 0.2 mg.kg^{-1} , followed by a constant infusion of $0.3 \text{ mg.kg}^{-1}.\text{h}^{-1}$.

The temperature of the animals was maintained using an under table heating device.

A neck dissection was performed and a stiff 16 gauge catheter inserted into the carotid artery and advanced to within 3cm of the aortic valve. This was connected to a transducer (Medex, Med Inc., UK) and used to display aortic systolic, diastolic and mean arterial pressures (SAP, DAP, MAP) continuously (Cardicap, Datex, Finland). The latter was obtained from the area under the aortic pressure recording. Lead I of the ECG was monitored continuously.

The right femoral area was dissected and an occlusion catheter (Fogarty, Baxter, USA) was inserted into the femoral artery and advanced into the descending thoracic aorta. This balloon was used to raise left ventricular (LV) afterload for the determination of end-systolic LV elastance (E_{es}) as described below.

A sternotomy was performed and the pericardium opened. A short stiff 14 gauge cannula, connected to a pressure transducer (Medex, Medex Ltd, UK) was inserted into the LV cavity via a stab incision in the LV apex. The catheter was sutured to the myocardium and was used to determine LV peak, LV end diastolic (LVEDP) and end-systolic pressures (PES), as well as the rate of pressure increase during isovolumic contraction ($+dp/dt$) and the rate of pressure decrease during isovolumic relaxation ($-dp/dt$). PES was taken at the time of the aortic valve closure (as judged from the dicrotic notch on the aortic pressure recording).

Two pairs of microsonometers were inserted into the LV subendocardium, one pair in the region supplied by the LAD artery and the second in the region

supplied by the circumflex coronary artery (LX), which was used as a control during the interventions. Signals were transduced (digital ultrasonic measurement system TRX series 8, Sonometrics Corporation, Canada) and the maximum segment length (L_{max}) at the end of diastole, as well as the minimum segment length at the end of systole (L_{min}), were recorded. The difference between L_{max} and L_{min} reflected regional segmental shortening (dL). Shortening that occurred after aortic valve closure was termed post-systolic shortening (PSS), and was expressed as a % of the total regional shortening (PSS%). This was an indication of early (isovolumic) diastolic dysfunction.

LV and regional length signals were combined to give beat-by-beat pressure-length loops. By raising the afterload with the aortic balloon, various pressure-length loops were recorded. The computer recognized the end-systolic pressure-length points of the various loops and performed a linear regression on these points. The slope of this was termed the end-systolic pressure-length relationship or end-systolic elastance (E_{es}), and was used as an index of regional myocardial contractility (Sagawa, 1997 and Kaseda 1985). The intercept of this regression on the length axis (x-axis) is the extrapolated regional segment length when the LV pressure is zero. This is the unstressed LV volume (L_0).

The product of the P_{es} and the dL , i.e. the area under the pressure-length loop (excluding the post-systolic shortening), is an index of regional strokework (RSW).

A one cm length of the proximal left anterior descending (LAD) coronary artery was mobilized from the underlying muscle. A soft silastic loop was placed around the artery. This was used to cause total occlusion of the LAD for 15 minutes as part of the experimental protocol. A laser doppler flow meter (small animal blood flow meter, T106, Transonic Systems Inc. New York, USA) was placed around the LAD just proximal to the silastic loop. This was to measure coronary artery blood flow. The LAD coronary artery was cannulated just distal to the silastic loop by means of a 26 gauge catheter (Abbocat-T, Abbott, Ireland), in order to administer drug or saline directly into the coronary artery. The coronary vein in the same region was cannulated by means of a 26 gauge catheter (Abbocat-T, Abbott, Ireland) in order to obtain venous blood gas samples from the affected area. Just before cannulation of the LAD, $1\text{mg}\cdot\text{kg}^{-1}$ heparin (Heparin, Intramed, SA) was given intravenously to prevent clot formation in the LAD artery during the period of occlusion.

All the transduced signals were digitized at a sampling rate of 100Hz by computer, running a custom-made program (ALAB, written by Ralph Pinna, Paul Bailey and Johan Coetzee, SED and the Department of Anesthesiology and Critical Care, University of Stellenbosch).

Data was recorded for 30 seconds at zero airway pressure and later redisplayed to evaluate the quality of the measurements.

During each recording blood samples were taken both from the proximal aorta and from the coronary vein. Analysis of blood samples was performed by easy blood gas blood analyzer (Medica, Bedford, Massachusetts). Blood lactate measurements were performed by a micro lactate analyzer (Akray Inc, Japan).

An initial dose finding protocol demonstrated that a dose of up to and including 2mg of verapamil administered intravenously over 8 minutes did not decrease mean arterial pressure by more than 10%.

Protocol

After completion of surgery, a 10 minute stabilization period was allowed, before control measurements were made (control). The study was then performed in **three sections**.

Section 1: No ischemia

Group 1: Verapamil 2mg

Section 2: Intervention 3 minutes before reperfusion.

Group 2: Verapamil 0.5mg.

Group 3: Verapamil 2 mg.

Group 4: Saline.

Section 3: Intervention at onset of ischemia.

Group 5: Verapamil 2mg.

Group 6: Saline

Section 1:

Group 1: (n = 6)

Verapamil was administered into the LAD coronary artery at $8\mu\text{g.kg}^{-1}.\text{min}^{-1}$ for 8 minutes (total dose \pm 2mg, total volume: 8 ml). Recordings were performed directly after the infusion ended, and after 10, 30, 60 and 90 minutes.

Sections 2 and 3:

Sections 2 and 3 are the ischemia models. In these groups the LAD coronary artery was occluded for 15 minutes. Recordings were made at 10 minutes after occlusion (ischemia), and after 10, 30, 60 and 90 minutes of reperfusion (reperfusion + 10 minutes; reperfusion + 30 minutes; reperfusion + 60 minutes and reperfusion + 90 minutes).

Group 2 (n = 8)

Three minutes before reperfusion (at 12 minutes of occlusion) an intracoronary verapamil infusion was started at $2\mu\text{g.kg}^{-1}.\text{min}^{-1}$ for 8 minutes i.e. 5 minutes into reperfusion (total dose \pm 0.5mg, total volume: 8ml).

Group 3 (n = 7)

Three minutes before reperfusion (at 12 minutes of occlusion) an intracoronary verapamil infusion was started at $8\mu\text{g.kg}^{-1}.\text{min}^{-1}$ for 8 minutes i.e. 5 minutes into reperfusion (total dose \pm 2mg, total volume: 8 ml).

Group 4 (n = 9)

Three minutes before reperfusion (at 12 minutes of occlusion) an intracoronary saline infusion was started at 1ml.min^{-1} for 8 minutes i.e. 5 minutes into reperfusion (total volume 8ml).

Group 5 (n = 6)

An intracoronary verapamil infusion was started at the onset of LAD coronary artery occlusion at $8\mu\text{g.kg}^{-1}.\text{min}^{-1}$ and continued for 8 minutes (total dose \pm 2mg, total volume: 8ml).

Group 6 (n = 6)

An intracoronary saline infusion was started at the onset of LAD coronary artery occlusion at 1ml.min^{-1} and continued for 8 minutes (total volume 8ml).

If more than three ventricular ectopic beats per minutes occurred, or if they were multifocal in origin, 1mg.kg^{-1} intravenous lidocaine was administered. This was repeated if necessary. The total dose of lidocaine required was recorded.

Ventricular tachycardia and ventricular fibrillation were treated with 1mg.kg^{-1} lidocaine intravenously, followed by direct defibrillation of the heart with 5, 5, 10, 10 and 15 Joules. The total amount of lidocaine and joules required to control the ventricular arrhythmias were recorded for each animal.

At the end of the experiment the heart was arrested with KCl intravenously while the animal was maintained under deep anesthesia. The heart was removed and the LAD artery cannulated with the tip of the cannula situated at the point where the artery had been occluded. Five ml of ink diluted with saline was injected into the coronary artery. The ventricles were opened and the area demarcated by the ink on the endocardium was excised from the heart. The mass of this area as well as the total LV mass were measured and recorded.

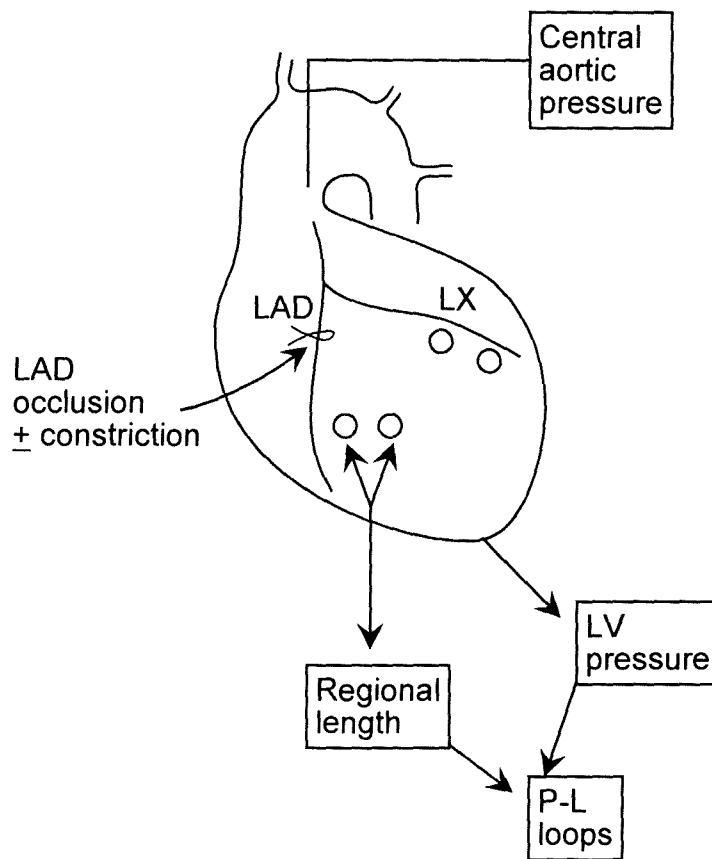


Fig. 2.1

A schematic drawing of the model used in this study. Two pairs of piezo-electric crystals were inserted into the subendocardium in areas supplied by the left descending (LAD) and circumflex (LX) coronary artery. LV pressure was obtained via a stiff cannula. The pressure and length signals were combined by computer and resulted in real time pressure-length loops. Aortic pressure was transduced from a catheter in the aorta. The position of the occlusion on the LAD is indicated.

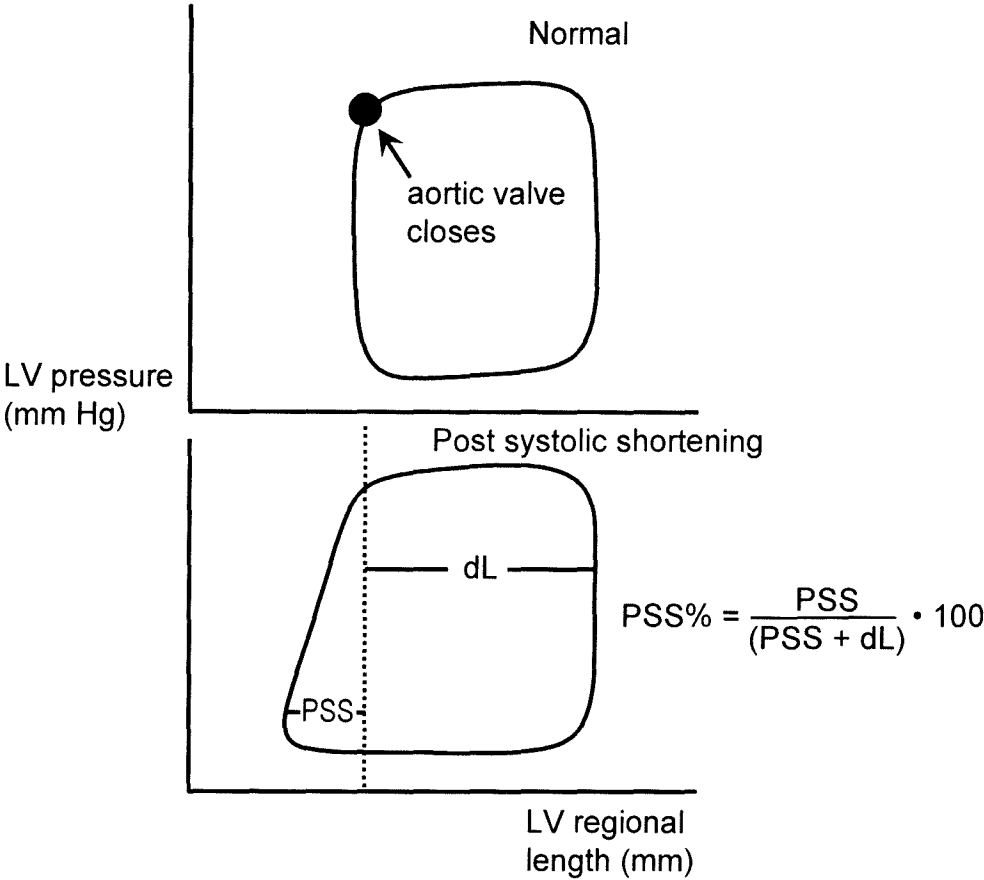


Fig. 2.2

The pressure length loop, demonstrating post-systolic shortening (PSS). This is shortening which occurs after the closure of the aortic valve and hence into early diastole. PSS does not contribute towards ejection. PSS is then expressed as a % of the total shortening i.e. the sum of dL and PSS.

A Coetzee

PhD (Stell) Effects of inhalation anesthesia on myocardial ischemia and reperfusion injury. University of Stellenbosch 1994

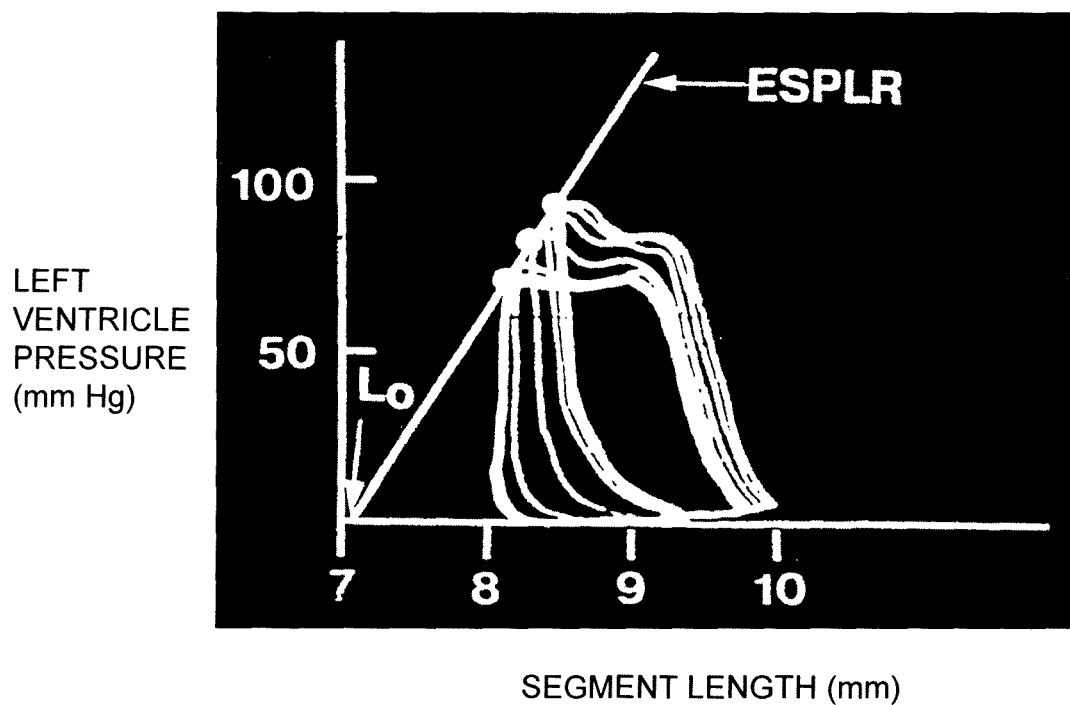


Fig 2.3

An example of pressure-length loops generated by altering the afterload. The end-systolic pressure and length points for the various afterloaded contractions were identified by computer and subjected to linear regression. The slope of this line is termed the end-systolic pressure-length relationship (ESPLR). It is also known as the E_{max} or E_{es} . It is regarded as a load independent index of regional myocardial contractility.

A Coetzee

PhD(Stell). Effect of inhalation anesthesia on myocardial ischemia and the reperfusion injury. University of Stellenbosch. 1994

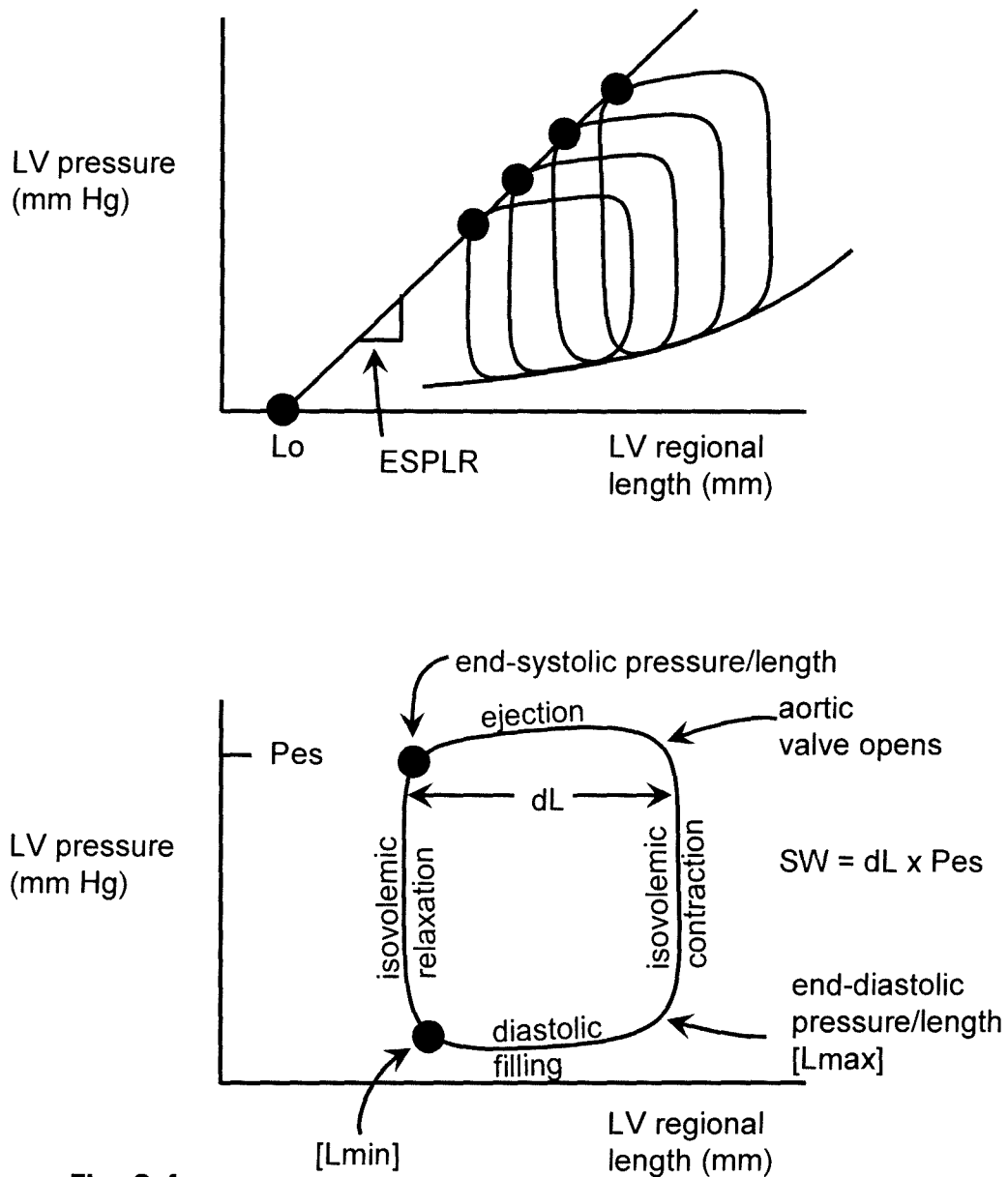


Fig. 2.4

Top: A schematic representation of regional pressure-length loops and the end-systolic pressure length relationship (ESPLR).

Bottom: The various components of the pressure-length loop depicting a single cardiac cycle. The product of regional shortening (dL) and end-systolic pressure (Pes) is the regional stroke work (SW).

A Coetzee

PhD(Stell). Effect of inhalation anesthesia on myocardial ischemia and reperfusion injury. University of Stellenbosch 1994

2.2 Measurements.

Total animal mass (kg)

Ischemic segment mass (g)

LV mass (g)

temperature (°C)

coronary bloodflow ($\text{mL} \cdot \text{min}^{-1}$)

heart rate

SAP (mmHg)

DAP (mmHg)

MAP (mmHg)

$+dp/dt_{\text{max}}$ ($\text{mm Hg} \cdot \text{s}^{-1}$)

$-dp/dt_{\text{max}}$ ($\text{mm Hg} \cdot \text{s}^{-1}$)

LAD Lmax: maximum LAD segment length (mm)

LAD Lmin₁ : LAD segment length when aortic valve closes (mm)

LAD Lmin₂ : minimum LAD segment length (mm)

LAD Lo : LAD segment length when LV pressure is zero (mm)

LAD Ees: LAD segment end-systolic elastance ($\text{mmHg} \cdot \text{mm}^{-1}$)

Pes: LV end-systolic pressure (mmHg)

Ped: LVEDP

LX Lmax: maximum LX segment length (mm)

LX Lmin₁: LX segment length when aortic valve closes (mm)

LX Lmin₂: minimum LX segment length (mm)

LX Lo : LX segment length when LV pressure is zero (mm)

LX Ees: LAD segment end-systolic elastance (mmHg.mm⁻¹)

Bloodgas measurements:

Arterial pH

venous pH

PaCO₂ (kPa)

PvCO₂ (kPa)

Pa O₂ (kPa)

PvO₂ (kPa)

HCO₃ (art) (mmol.l⁻¹)

HCO₃ (ven) (mmol.l⁻¹)

SaO₂ (%)

SvO₂ (%)

Arterial lactate (mmol.l⁻¹)

Venous lactate (mmol.l⁻¹)

Haemoglobin (g.dl⁻¹)

2.3 Calculations:

LAD tot dL : total shortening of the LAD segment (mm)

$$\text{LAD Lmax} - \text{LAD Lmin}_2$$

LAD syst dL : total systolic shortening of the LAD segment (mm)

$$\text{LAD Lmax} - \text{LAD Lmin}_1$$

LAD syst dL%: LAD segment systolic shortening as a percentage of maximum LAD segment length (%)

$$\text{LAD syst dL} / \text{LAD Lmax} \times 100$$

LAD PSS : LAD segment post-systolic shortening (mm)

$$\text{LAD Lmin}_1 - \text{LAD Lmin}_2$$

LAD PSS% : LAD segment post-systolic shortening as a percentage of total LAD segment shortening(%)

$$\text{LAD PSS} / \text{LAD tot dL} \times 100$$

LAD SW : LAD segment stroke work (mm.mmHg)

$$\text{LAD syst dL} \times \text{Pes}$$

LAD stat C : LAD static compliance (mm.mmHg⁻¹)

$$\text{LAD Lmax} / \text{LVEDP}$$

LX tot dL	:	Total shortening of LX segment (mm) $LX\ L_{max} - LX\ L_{min_2}$
LX syst dL:	:	systolic shortening of LX segment (mm) $LX\ L_{max} - Lx\ L_{min_1}$
LX Syst dL%:	:	LX segment systolic shortening as a percentage of maximum LX segment length (%) $LX\ syst\ dL / LX\ L_{max} \times 100$
LX PSS	:	LX segment post-systolic shortening (mm) $LX\ L_{min_1} - LX\ L_{min_2}$
LX PSS%	:	LX segment post-systolic shortening as a percentage of total LX segment shortening (%) $LX\ PSS / LX\ tot\ dL \times 100$
LS SW	:	LX segment stroke work (mm.mmHg) $LX\ syst\ dL \times P_{es}$
LX stat C	:	LX segment static compliance (mm.mmHg ⁻¹) $LX\ L_{max} / LVEDP$
CaO ₂	:	Arterial oxygen content (ml/100ml)

$$(1.39 \times (\text{SaO}_2/100) \times \text{Hb}) + (0.0031 \times \text{PaO}_2 \times 7.5006)$$

CvO_2 : Coronary venous oxygen content (ml/100ml)

$$(1.39 \times (\text{SVO}_2/100) \times \text{Hb}) + (0.0031 \times \text{PvO}_2 \times 7.5006)$$

a-v dO_2 : Arterial-venous oxygen content difference (ml/100ml)

$$\text{CaO}_2 - \text{CvO}_2$$

$\text{a-v dO}_2\%$: Arterial-venous oxygen content difference as a percentage of arterial oxygen content (%).

$$(\text{CaO}_2 - \text{CvO}_2) / \text{CaO}_2 \times 100$$

$\dot{\text{V}}\text{O}_2$: Oxygen consumption in the ischemic segment (ml.min⁻¹)

$$\text{cbf} (\text{CaO}_2 - \text{CvO}_2) / 100$$

$\text{DO}_2: \dot{\text{V}}\text{O}_2$: ratio of oxygen delivery to oxygen consumption

$$(\text{cbf}/100\text{g}) / \dot{\text{V}}\text{O}_2 100\text{g}$$

a-v dlact : difference in arterial and venous lactate (mmol.l⁻¹)

$$\text{art lact} - \text{ven lact}$$

2.4 Statistical analysis

The statistical package Sigmastat en Sigmaplot (SPSS Inc., Chicago, Illinois, USA. 1997) was used in the analysis of data.

Descriptive statistics included the mean and standard deviation of the mean.

For analysis of data within groups (various experimental sections), one way repeated measures analysis of variance was used. Data was tested for normality and equal variance. If the test passed the normality test, specific differences between various steps were sought with a multiple comparison procedure (Turkey Test). If normality failed, repeated measures analysis of variance based on ranks was done.

The power of the test was also evaluated and considered in the results obtained. The desired power was set at 0.8.

A probability of exceeding .05 was accepted as indicative of statistically significant differences between steps.

Each step was compared to own control (initial) value. The *time* at which a specific parameter recovered to the initial (control) value was noted. Comparison *between the various protocols* was then further considered with

reference to the *time it took to recover* to control value and this difference was then used in further deductions and discussions.

For comparison of the incidence of arrhythmias and defibrillation requirements, Chi^2 was applied.

In the following section, in the comments on results, any stated increase or decrease in any value at any point in time, means a statistically significant change.

3. Results

- 3.1 Group 1: Verapamil 2 mg ($8\mu\text{g.kg}^{-1}.\text{min}^{-1}$) in the nonischemic heart.**
- 3.2 Group 2: Verapamil 0.5mg ($2\mu\text{g.kg}^{-1}.\text{min}^{-1}$) started 3 minutes before reperfusion and continued for 8 minutes.**
- 3.3 Group 3: Verapamil 2mg ($8\mu\text{g.kg}^{-1}.\text{min}^{-1}$) started 3 minutes before reperfusion and continued for 8 minutes.**
- 3.4 Group 4: Saline infusion started 3 minutes before reperfusion and continued for 8 minutes.**
- 3.5 Group 5: Verapamil 2 mg ($8\mu\text{g.kg}^{-1}.\text{min}^{-1}$) started at the onset of ischemia and continued for 8 minutes.**
- 3.6 Group 6: Saline infusion started at the onset of ischemia and continued for 8 minutes.**
- 3.7 Reperfusion arrhythmias.**
- 3.8 Evidence of ischemia.**
 - 3.8.1 Myocardial function.**
 - 3.8.2 Myocardial metabolism.**

General comments.

The 42 pigs enrolled in the study all had similar mass, temperature, mean arterial pressure and coronary bloodflow at the onset of the experiments. There was also no difference in ischemic segment mass or total LV mass between the groups.

Of the 42 pigs, 6 received 2 mg of intracoronary verapamil in the absence of ischemia (section 1, group 1). The remaining 36 animals were randomized to the 5 experimental groups in sections 2 and 3. Three pigs died during the experiments due to refractory ventricular fibrillation on reperfusion, two in the saline groups and one in the low dose (0.5mg) verapamil group.

For clarity the sections and groups are again listed:

Section 1 – no ischemia.

Group 1 – verapamil 2mg.

Section 2 – interventions 3 minutes before reperfusion.

Group 2 – 0.5mg verapamil over 8 minutes.

Group 3 – 2 mg verapamil over 8 minutes.

Group 4 – saline infusion for 8 minutes.

Section 3 – interventions at the onset of ischemia.

Group 5 – 2 mg verapamil over 8 minutes.

Group 6 – saline infusion for 8 minutes.

3.1 Group 1: Verapamil 2mg ($8\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) in the non ischemic heart

3.1.1 General data

Step	Tot mass	Isch mass	Vent mass	Temp	CBF	HR	MAP	Pes	Ped	+dp/dt	-dp/dt
Control	33.167 4.021	45.005 10.506	107.038 21.723	37.733 0.977	26.333 10.820	101.167 14.331	83.000 18.330	90.098 22.921	12.057 1.438	1311.760 165.641	1351.490 292.899
Post infusion	33.167 4.021	45.005 10.506	107.038 21.723	37.717 1.098	75.500 20.686	100.000 14.311	66.333 10.690	70.137 13.865	14.333 6.631	1012.012 155.171	1157.550 188.633
Infusion + 10 min	33.167 4.021	45.005 10.506	107.038 21.723	37.700 1.135	54.167 18.159	95.833 17.440	69.500 11.777	74.648 16.668	13.518 4.198	1021.193 184.295	1207.047 192.541
Infusion + 30 min	33.167 4.021	45.005 10.506	107.038 21.723	37.617 1.230	31.000 6.512	88.000 16.876	79.833 13.393	89.697 17.629	13.086 5.721	1223.412 208.097	1408.803 171.166
Infusion + 60 min	33.167 4.021	45.005 10.506	107.038 21.723	37.467 1.397	32.500 5.577	87.333 16.379	86.833 11.548	99.235 16.590	13.648 6.723	1254.082 110.217	1432.878 193.852
Infusion + 90 min	33.167 4.021	45.005 10.506	107.038 21.723	37.433 1.477	31.500 5.010	87.000 14.656	91.000 16.637	102.658 20.505	16.108 4.690	1238.985 174.998	1433.370 313.240

Tot mass – total animal mass (kg)

Isch mass – ischemic segment mass (g)

Vent mass – left ventricle mass (g)

Temp – temperature ($^{\circ}\text{C}$)

CBF – coronary bloodflow (mL/min)

HR – heart rate (min^{-1})

MAP – mean arterial pressure (mmHg)

+dp/dt – maximal positive value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

-dp/dt – maximal negative value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

Pes – left ventricular pressure at the end of systole (mmHg)

Ped – left ventricular pressure at the end of diastole (mmHg)

Coronary blood flow increased 3 fold upon administration of intracoronary verapamil and remained higher than baseline throughout the subsequent 90 minutes. However 10 minutes after the infusion it had decreased to twice

baseline values and 30 min after the infusion CBF was 15% higher than baseline. It remained at this level for the rest of the experiment.

Heart rate remained constant and similar to control values until 10 minutes after the verapamil infusion. At 30 minutes after the verapamil infusion, heart rate was decreased and it remained decreased at the same level for the remainder of the experiment.

MAP decreased when verapamil was infused, remained decreased 10 minutes after the infusion, but had returned to control values by 30 minutes after the infusion where it remained constant for the rest of the experiment.

dP/dt decreased on verapamil infusion and remained decreased 10 minutes after the infusion. By 30 minutes after infusion dP/dt had normalized to control values, and remained constant for the rest of the experiment.

-dP/dt: At 60 and 90 minutes post infusion the -dP/dt was higher than the value directly after the verapamil infusion, but no difference from the control value.

LV end systolic pressure decreased after verapamil infusion, but returned to control values by 30 minutes after the infusion.

LV end diastolic pressure did not change during the experiment.

3.1.2 Segment data

Step	LAD Lmax	LAD Lmin ₁	LAD Lmin ₂	LAD L ₀	LAD Ees	LxLmax	LxLmin ₁	LxLmin ₂	Lx L ₀	LX Ees
Control	19.158 3.678	14.820 3.181	14.513 3.299	10.627 2.942	37.105 22.397	14.575 2.954	11.180 2.078	11.180 2.078	9.198 3.864	52.715 22.752
Post infusion	20.803 3.278	18.120 2.618	16.848 3.217	15.023 2.278	32.088 9.129	15.143 3.296	11.933 2.468	11.893 2.461	10.047 2.000	47.808 18.764
Infusion + 10 min	20.048 3.420	16.695 2.568	15.805 2.902	12.708 2.468	26.768 11.175	14.563 2.974	11.558 2.017	11.475 2.007	9.700 2.199	48.108 24.227
Infusion + 30 min	17.878 4.708	14.727 2.576	14.607 2.791	11.020 2.470	29.612 12.955	14.683 3.134	11.492 2.127	11.385 2.129	9.328 1.881	53.327 26.633
Infusion + 60 min	18.977 3.273	14.407 2.783	14.407 2.783	9.772 3.428	31.455 18.931	14.527 3.094	11.322 2.071	11.238 2.079	8.972 1.639	53.245 27.251
Infusion + 90 min	18.925 3.258	14.512 2.607	14.465 2.685	10.488 2.798	32.187 19.635	14.513 3.080	11.483 2.184	11.417 2.190	9.505 2.010	96.090 118.813

LAD Lmax – maximum LAD segment length (mm)

LAD Lmin₁ – minimum LAD segment length at the end of systole (mm)

LAD Lmin₂ – minimum LAD segment length (mm)

LAD L₀ – LAD segment length when left ventricular pressure is zero (mm)

LAD Ees – end systolic elastance of the LAD segment (mmHg.mm⁻¹)

LX Lmax – maximum circumflex segment length (mm)

LX Lmin₁ – minimum circumflex segment length at the end of systole (mm)

LX Lmin₂ – minimum circumflex length (mm)

LX L₀ – circumflex segment length when left ventricular pressure is zero (mm)

LX Ees – end systolic elastance of the circumflex segment (mmHg.mm⁻¹)

LAD segment

Maximum LAD segment length (LAD Lmax) increased directly after verapamil infusion, but returned to control values by 30 minutes after the infusion.

LAD Lo (the unstressed LAD segment length) increase directly after the verapamil infusion, but had returned to control values by 10 minutes after the infusion.

LAD segment end systolic elastance (index of contractility) remained unaffected by verapamil infusion.

Circumflex segment (LX)

Maximum LX segment length was unaffected by verapamil infusion, as was LX Lo and LX ees.

3.1.3 LAD segment calculations

Step	LADtot dL	LAD syst dL	LAD syst dL %	LAD PSS	LAD PSS %	LAD SW	LAD stat C
Control	4.645	4.338	22.900	0.307	6.478	389.338	1.630
	0.635	0.722	3.275	0.522	11.192	114.992	0.475
Post infusion	3.955	2.683	12.618	1.272	32.428	194.313	1.812
	0.663	1.136	4.438	1.002	23.565	110.183	1.012
Infusion + 10 min	4.243	3.353	16.700	0.890	19.430	260.240	1.785
	0.940	1.106	3.420	0.604	16.904	126.590	0.825
Infusion + 30 min	3.272	3.152	13.609	0.120	2.934	265.727	1.617
	3.644	3.623	25.461	0.294	7.187	354.240	0.899
Infusion + 60 min	4.570	4.570	24.295	0.000	0.000	453.759	1.757
	0.812	0.812	3.554	0.000	0.000	119.440	1.022
Infusion + 90 min	4.460	4.413	23.383	0.047	1.296	451.747	1.288
	0.780	0.848	2.607	0.114	3.175	125.789	0.517

LAD tot dL – total shortening of LAD segment (mm)

LAD syst dL – total systolic shortening of LAD segment (mm)

LAD syst dL% - LAD segment systolic shortening as a percentage of maximum LAD segment length (%)

LAD PSS – LAD segment postsystolic shortening (mm)

LAD PSS% - LAD segment postsystolic shortening as a percentage of total LAD segment shortening (%)

LAD SW – LAD segment stroke work (mm.mmHg)

LAD stat C – LAD segment static compliance (mm.mmHg⁻¹)

Total LAD segment shortening (LAD tot dL), total systolic shortening (LAD syst dL), and LAD segment systolic shortening as a percentage of maximum LAD segment length (LAD syst dL%) increased 60 minutes after verapamil infusion compared to directly after the infusion, but were not greater than the control values.

LAD segment post-systolic shortening (LAD PSS) decreased at 30 minutes after the infusion, compared to directly after the infusion, to values no different from controls.

LAD segment postsystolic shortening as a percentage of total LAD segment shortening (LAD PSS%), increased directly after the verapamil infusion, but returned to control values 30 minutes after the infusion.

LAD segment stroke work (LAD SW) increased at 60 minutes after the verapamil infusion and remained increased (compared to directly after the infusion) at values no different from control values.

LAD segment static compliance (LAD stat C) remained unchanged throughout the experiment.

3.1.4 Circumflex segment calculations

Step	LX tot dL	LX syst dL	LX syst dL%	LX PSS	LX PSS%	LX SW	LX stat C
Control	3.395	3.395	22.991	0.000	0.000	304.094	1.217
	1.097	1.097	4.809	0.000	0.000	110.656	0.245
Post infusion	3.250	3.210	20.926	0.040	1.961	226.815	1.201
	1.288	1.336	7.122	0.098	4.803	101.972	0.416
Infusion + 10 min	3.088	3.005	20.051	0.083	3.374	223.571	1.131
	1.280	1.343	7.191	0.204	8.264	97.830	0.311
Infusion + 30 min	3.298	3.192	21.285	0.107	3.980	289.912	1.267
	1.125	1.222	4.898	0.261	9.749	129.226	0.501
Infusion + 60 min	3.288	3.205	21.554	0.083	3.098	321.213	1.288
	1.182	1.248	5.157	0.204	7.588	140.708	0.657
Infusion + 90 min	3.097	3.030	20.452	0.067	2.699	313.160	0.935
	1.097	1.154	5.020	0.163	6.611	144.499	0.184

LX tot dL – LX segment total shortening (mm)

LX syst dL – LX segment total systolic shortening (mm)

LX syst dL% - LX segment systolic shortening as a percentage of maximum LX segment length (%)

LX PSS – LX segment post systolic shortening (mm)

LX PSS% - LX segment post systolic shortening as a percentage of total LX segment shortening (%)

LX SW – LX segment stroke work (mm.mmHg)

LX stat C – LX segment static compliance (mm.mmHg⁻¹)

There were no changes in: LX segment total shortening (Lx tot dL), LX segment systolic shortening (LX syst dL) or LX segment systolic shortening as a

percentage of maximum LX segment length (LX syst dL%) during the experiment.

There were no changes in LX segment post systolic shortening (LXPSS), or LX segment postsystolic shortening as a percentage of total LX segment shortening LX PSS% during the experiment. There was also no change in LX segment static compliance (LX stat C) throughout the experiment.

3.1.5 Bloodgas data

Step	pH a	PaCO ₂	PaO ₂	HCO ₃ a	SaO ₂	Hb	pH v	PvCO ₂	PvO ₂	HCO ₃ v	SvO ₂	lac a	lac v
Control	7.418	5.260	49.033	28.533	99.933	10.717	7.333	7.490	3.450	30.400	43.117	0.533	0.617
	0.026	0.731	17.332	1.496	0.082	1.744	0.097	2.227	0.489	1.491	7.021	0.480	0.646
Post infusion	7.413	5.312	49.500	28.917	99.950	10.667	7.385	5.488	9.567	27.850	94.050	0.567	0.683
	0.021	0.679	13.255	1.685	0.084	1.780	0.016	0.622	1.776	1.359	2.944	0.513	0.668
Infusion + 10 min	7.388	5.552	37.967	27.900	99.833	10.667	7.365	6.368	6.283	29.667	82.083	0.583	0.617
	0.016	0.755	14.610	1.207	0.103	1.780	0.018	1.022	0.325	1.524	3.338	0.531	0.624
Infusion + 30 min	7.433	5.407	45.833	28.283	99.833	10.750	7.363	6.690	4.083	30.200	56.033	0.567	0.533
	0.083	1.018	20.707	1.474	0.225	1.782	0.039	1.454	0.671	2.252	12.857	0.520	0.493
Infusion + 60 min	7.398	5.610	48.800	28.650	99.883	10.750	7.367	6.315	4.283	29.633	61.150	0.950	0.583
	0.018	0.769	18.902	1.495	0.117	1.782	0.025	1.128	0.387	2.024	10.075	0.826	0.875
Infusion + 90 min	7.412	5.303	46.700	28.233	99.883	10.750	7.368	6.627	3.650	30.167	50.017	0.733	0.667
	0.010	0.988	20.479	1.760	0.147	1.782	0.032	1.425	0.152	2.199	5.406	0.509	0.745

pH a – arterial pH

PaCO₂ – arterial PCO₂ (kPa)

Pa O₂ – arterial PO₂ (kPa)

HCO₃a – arterial HCO₃ (mmol.l⁻¹)

SaO₂ – arterial saturation (%)

Hb – hemoglobin (g.dl⁻¹)

pH v – coronary venous pH

PvCO₂ – coronary venous PCO₂ (kPa)

PvO₂ – coronary venous PO₂ (kPa)

HCO_3^- v – coronary venous HCO_3^- (mmol. ℓ^{-1})

SvO_2 – coronary venous saturation (%)

lac a – arterial lactate (mmol. ℓ^{-1})

lac v – coronary venous lactate (mmol. ℓ^{-1})

Bloodgas data

Hb and venous lactate remained constant throughout the experiment.

3.1.6 Bloodgas calculation

Step	CaO_2	CvO_2	a-v dO_2	a-v $\text{dO}_2\%$	$\dot{\text{V}}\text{O}_2$	$\dot{\text{V}}\text{O}_2/100\text{g}$	$\dot{\text{V}}\text{O}_2/100\text{g/beat}$	CBF/100g	CBF/100g/beat	a – v d lact
Control	16.027 2.702	6.542 1.730	9.485 1.747	59.332 6.773	2.483 1.159	6.013 3.890	0.063 0.048	61.379 31.110	0.639 0.384	-0.083 0.371
Post infusion	15.971 2.640	14.201 2.550	1.770 0.300	11.297 2.549	1.346 0.503	3.160 1.494	0.032 0.016	180.923 81.623	1.901 1.098	-0.117 0.240
Infusion + 10 min	15.684 2.395	12.346 2.292	3.338 0.553	21.565 3.776	1.763 0.452	4.070 1.360	0.045 0.021	124.818 47.627	1.354 0.586	-0.033 0.301
Infusion + 30 min	15.982 2.673	8.629 3.257	7.354 1.704	47.052 11.613	2.208 0.366	5.080 1.278	0.062 0.030	69.900 11.221	0.821 0.228	0.033 0.513
Infusion + 60 min	16.060 2.509	9.169 1.907	6.891 1.692	42.743 8.076	2.174 0.362	5.141 1.701	0.062 0.030	74.830 18.190	0.897 0.351	0.367 0.484
Infusion + 90 min	16.011 2.659	7.535 1.423	8.476 1.623	52.794 4.869	2.680 0.730	6.347 2.546	0.076 0.038	72.872 19.339	0.866 0.322	0.067 0.774

CaO_2 – arterial oxygen content (mL.100mL $^{-1}$)

CvO_2 – coronary venous oxygen content (mL.100mL $^{-1}$)

a-v dO_2 – arterial-coronary venous oxygen content difference (mL.100mL $^{-1}$)

a-v $\text{dO}_2\%$ – arterial-coronary venous oxygen content difference as a percentage of arterial oxygen content (%)

$\dot{\text{V}}\text{O}_2$ – oxygen consumption in LAD segment (mL.min $^{-1}$)

$\dot{\text{V}}\text{O}_2/100\text{g}$ – oxygen consumption per 100g LAD segment tissue (mL.min $^{-1}$)

$\dot{\text{V}}\text{O}_2/100\text{g/beat}$ – oxygen consumption per 100g LAD segment tissue per beat (mL)

CBF/100g – coronary blood flow per 100g LAD segment tissue (mL.min $^{-1}$)

CBF/100g/beat – coronary blood flow per 100g LAD segment tissue per beat (mL)

a-v d lact – difference in arterial and venous lactate (mmol. ℓ^{-1})

Arterial oxygen content remained constant throughout the experiment.

VO_2 and $VO_2/100g$ LAD segment tissue in the LAD segment, decreased directly after the verapamil infusion. Ten minutes after the infusion the VO_2 was again no different from control values, and at 90 minutes after the infusion VO_2 was significantly higher than directly after the infusion, but no different from the control value.

$VO_2/100g/beat$ decreased directly after the infusion. Ten minutes after the infusion there was again no difference from control values, and at 90 minutes after the infusion the $VO_2/100g/beat$ was significantly higher than directly after the infusion, but no different from control values.

CBF/100g in the LAD segment increased directly after the verapamil infusion and had returned to control values by 30 minutes after the infusion. By 10 minutes after the infusion CBF/100g was already significantly lower than directly after the infusion, but still higher than control values.

CBF/100g/beat in the LAD segment increased directly after the verapamil infusion, and had returned to control values by 30 minutes after the infusion.

The difference in arterial and venous lactate (a-v d lact) remained unchanged throughout the experiment.

3.2 Group 2: Verapamil 0.5mg ($2\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) started 3 minutes before reperfusion and continued for 8 minutes

3.2.1 General data

Step	Total mass	Isch mass	Vent mass	Temp	CBF	HR	MAP	Pes	Ped	+dP/dt	- dP/dt
Control	28.857 2.035	38.877 8.005	96.185 18.271	37.314 0.953	33.857 8.395	105.000 26.245	70.667 12.144	74.905 15.984	10.302 4.487	1205.500 388.125	1202.190 160.434
Isch	28.857 2.035	38.877 8.005	96.185 18.271	37.143 0.971	2.743 3.010	105.143 24.464	65.857 15.214	69.060 20.105	13.863 5.143	1077.020 323.828	1076.121 254.895
Rep + 10 min	28.857 2.035	38.877 8.005	96.185 18.271	36.971 0.948	64.571 22.337	101.429 29.748	65.286 15.141	70.853 21.107	13.251 4.417	959.579 200.136	1018.060 359.056
Rep + 30 min	28.857 2.035	38.877 8.005	96.185 18.271	36.671 0.780	34.714 12.593	98.143 21.629	76.000 12.490	84.221 15.791	13.980 4.396	1036.436 231.314	1234.764 240.208
Rep + 60 min	28.857 2.035	38.877 8.005	96.185 18.271	36.300 0.627	35.000 12.247	100.857 24.162	73.571 12.354	80.041 17.264	10.981 4.755	1134.451 354.026	1145.069 226.379
Rep + 90 min	28.857 2.035	38.877 8.005	96.185 18.271	36.300 0.661	32.714 10.259	101.000 25.443	75.000 8.794	84.561 11.618	11.430 4.962	1127.013 412.008	1104.667 118.521

Tot mass – total animal mass (kg)

Isch mass – ischemic segment mass (g)

Vent mass – left ventricle mass (g)

Temp – temperature ($^{\circ}\text{C}$)

CBF – coronary bloodflow (mL/min)

HR – heart rate (min^{-1})

MAP – mean arterial pressure (mmHg)

+dP/dt – maximal positive value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

-dP/dt – maximal negative value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

Pes – left ventricular pressure at the end of systole (mmHg)

Ped – left ventricular pressure at the end of diastole (mmHg)

CBF decreased at the onset of ischemia, but had returned to control values by 10 minutes after reperfusion.

Heart rate and MAP remained constant throughout the experiment.

dP/dt and $-dP/dt$ were both decreased at 10 minutes after reperfusion compared to control values. However both had returned to control values by 30 minutes after reperfusion.

LV endsystolic pressure was increased at 30 and 90 minutes post reperfusion, compared to ischemic values, and the values at 10 minutes post reperfusion.

LV endsystolic pressure did not change during the experiment.

3.2.2 Segment data

Step	LAD Lmax	LAD Lmin ₁	LAD Lmin ₂	LAD L ₀	Lad Ees	LXLmax	LXLmin ₁	LXLmin ₂	LX L ₀	LX Ees
Control	20.813 0.687	15.777 1.622	15.590 1.536	12.693 1.935	30.413 15.061	14.833 4.772	11.483 3.436	11.483 3.436	10.878 3.223	65.177 69.968
Isch	22.949 1.433	21.656 1.800	19.723 1.489	18.907 1.785	37.084 19.677	15.917 4.305	12.677 3.464	12.677 3.464	12.844 6.043	48.679 22.442
Rep + 10 min	22.386 1.634	19.646 1.896	18.623 1.730	16.049 3.118	24.490 8.165	15.490 4.675	12.799 3.554	12.799 3.554	11.069 3.998	42.104 15.839
Rep + 30 min	21.927 1.675	18.651 1.961	17.911 1.827	13.723 2.421	20.174 11.391	15.486 4.484	12.636 3.441	12.607 3.479	12.024 2.726	80.540 81.416
Rep + 60 min	20.779 1.375	17.453 1.958	17.149 1.979	12.644 2.714	25.841 26.883	15.080 4.408	12.461 3.276	12.464 3.281	9.84 3.36	40.441 20.734
Rep + 90 min	20.520 1.589	17.323 2.014	17.014 1.971	11.204 3.198	24.164 33.653	14.864 4.355	12.620 3.515	12.620 3.515	10.320 3.299	57.041 40.665

LAD Lmax – maximum LAD segment length (mm)

LAD Lmin₁ – minimum LAD segment length at the end of systole (mm)

LAD Lmin₂ – minimum LAD segment length (mm)

LAD L₀ – LAD segment length when left ventricular pressure is zero (mm)

LAD Ees – end systolic elastance of the LAD segment (mmHg.mm⁻¹)

LX Lmax – maximum circumflex segment length (mm)

LX Lmin₁ – minimum circumflex segment length at the end of systole (mm)

LX Lmin₂ – minimum circumflex length (mm)

LX L₀ – circumflex segment length when left ventricular pressure is zero (mm)

LX Ees – end systolic elastance of the circumflex segment (mmHg.mm⁻¹)

LAD segment

Maximum LAD segment length (LAD L max) increased during ischemia and remained increased at 10 and 30 minutes reperfusion. LAD L max returned to control values by 60 min reperfusion.

LAD Lo (the unstressed LAD segment length) increased during ischemia remained unchanged from this value at 10 minutes reperfusion, but had normalized to control values by 30 minutes reperfusion.

LD segment endsystolic elastance (Ees) remained unchanged.

Circumflex segment (LX):

LX L max, LX Lo and LX Ees remained unchanged during the experiment.

3.2.3 LAD segment calculations

Step	LADtot dL	LAD syst dL	LAD syst dL %	LAD PSS	LAD PSS %	LAD SW	LAD stat C
Control	5.223	5.037	24.315	0.187	3.719	377.632	2.294
	0.943	1.091	5.801	0.457	9.108	107.790	0.766
Isch	3.226	1.293	5.656	1.933	61.974	98.016	1.881
	0.697	1.015	4.413	0.808	26.319	93.784	0.757
Rep + 10 min	3.763	2.740	12.265	1.023	26.153	196.751	1.859
	0.887	1.106	4.740	1.083	24.855	94.366	0.686
Rep + 30 min	4.016	3.276	15.047	0.740	19.440	276.075	1.717
	0.824	0.889	4.363	0.288	10.165	96.299	0.616
Rep + 60 min	3.630	3.326	16.175	0.304	8.039	267.666	2.167
	0.954	0.926	4.907	0.437	10.403	100.657	0.788
Rep + 90 min	3.506	3.197	15.701	0.309	9.389	269.487	2.170
	1.093	1.057	5.505	0.336	8.591	96.085	1.123

LAD tot dL – total shortening of LAD segment (mm)

LAD syst dL – total systolic shortening of LAD segment (mm)

LAD syst dL% - LAD segment systolic shortening as a percentage of maximum LAD segment length (%)

LAD PSS – LAD segment postsystolic shortening (mm)

LAD PSS% - LAD segment postsystolic shortening as a percentage of total LAD segment shortening (%)

LAD SW – LAD segment stroke work (mm.mmHg)

LAD stat C – LAD segment static compliance (mm.mmHg⁻¹)

LAD segment calculations

Total LAD segment shortening decreased during ischemia and remained decreased compared to the control value for the entire 90 minutes after reperfusion.

Systolic shortening of the LAD segment (LAD syst dL) decreased during ischemia and remained decreased compared to the control value for the entire 90 minutes post reperfusion. The systolic shortening during reperfusion was however consistently greater than that during ischemia.

LAD segment systolic shortening as a percentage of maximum LAD segment length decrease during ischemia and remained decreased compared to the control value for the entire 90 minutes post reperfusion. However from 30 minutes post reperfusion the value was consistently greater than that during ischemia.

LAD segment postsystolic shortening (LAD PSS) increased during ischemia, remained increased at 10 minutes post reperfusion, and returned to control values by 30 minutes post reperfusion.

LAD segment postsystolic shortening as a percentage of total LAD segment shortening increased during ischemia, and returned to control values by 10 minutes post reperfusion.

LAD segment stroke work decreased during ischemia, remained decreased at 10 minutes post reperfusion, and returned to control values by 30 minutes post reperfusion.

LAD segment static compliance remained unchanged throughout.

3.2.4 Circumflex segment calculations

Step	LX tot dL	LX syst dL	LX syst dL%	LX PSS	LX PSS%	LX SW	LX stat C
Control	3.350	3.350	21.802	0.000	0.000	258.585	1.630
	1.962	1.962	8.303	0.000	0.000	171.370	0.708
Isch	3.240	3.240	20.253	0.000	0.000	235.241	1.408
	1.671	1.671	8.233	0.000	0.000	141.010	0.915
Rep + 10 min	2.691	2.691	16.666	0.000	0.000	205.530	1.301
	1.640	1.640	7.265	0.000	0.000	149.627	0.555
Rep + 30 min	2.879	2.850	17.904	0.029	1.832	240.307	1.244
	1.450	1.482	6.134	0.076	4.846	127.711	0.598
Rep + 60 min	2.616	2.619	16.651	-0.003	-0.055	214.421	1.674
	1.534	1.540	6.376	0.008	0.145	134.224	0.931
Rep + 90 min	2.244	2.244	14.649	0.000	0.000	194.309	1.597
	1.292	1.292	6.195	0.000	0.000	117.772	0.941

LX tot dL – LX segment total shortening (mm)

LX syst dL – LX segment total systolic shortening (mm)

LX syst dL% - LX segment systolic shortening as a percentage of maximum LX segment length (%)

LX PSS – LX segment post systolic shortening (mm)

LX PSS% - LX segment post systolic shortening as a percentage of total LX segment shortening (%)

LX SW – LX segment stroke work (mm.mmHg)

LX stat C – LX segment static compliance (mm.mmHg⁻¹)

By 60 minutes post reperfusion the LX segment total shortening and the LX segment systolic shortening had decreased compared to control values, as had the LX segment systolic shortening as a percentage of maximum LX segment length.

There were no changes in LX segment post systolic shortening (LX PSS), or LX segment postsystolic shortening as a percentage of total LX segment shortening

LX PSS% during the experiment. There was also no change in LX stat C during the experiment.

3.2.5 Bloodgas data

Step	pH a	PaCO ₂	PaO ₂	HCO _{3a}	SaO ₂	Hb	pH v	PvCO ₂	PvO ₂	HCO _{3v}	SvO ₂	lac a	lac v
Control	7.414 0.050	6.070 0.617	38.886 16.555	29.257 2.231	99.771 0.315	10.357 0.748	7.341 0.048	8.004 0.626	3.214 0.573	32.586 2.527	37.557 10.796	0.500 0.374	0.283 0.390
Isch	7.401 0.041	5.781 0.962	38.443 16.503	28.029 1.986	99.800 0.173	10.357 0.748	7.201 0.104	10.417 3.532	3.714 1.671	29.571 3.182	37.129 23.763	0.400 0.374	4.314 2.769
Rep + 10 min	7.384 0.036	6.044 0.680	32.400 10.704	27.943 2.076	99.686 0.313	10.500 0.764	7.359 0.035	6.533 0.651	6.529 1.106	28.814 2.265	81.443 8.425	1.100 1.449	1.211 1.895
Rep + 30 min	7.397 0.034	5.727 0.751	31.814 12.681	27.329 2.105	99.729 0.214	10.429 0.732	7.361 0.031	6.640 0.669	4.171 1.097	29.057 1.984	54.843 14.662	0.943 0.905	0.983 0.978
Rep + 60 min	7.399 0.024	5.779 0.616	29.457 6.463	28.029 2.468	99.729 0.189	10.429 0.732	7.354 0.029	6.959 0.642	3.929 1.179	25.954 8.547	50.171 15.566	0.814 0.641	0.926 0.908
Rep + 90 min	7.403 0.028	5.559 0.500	31.714 13.098	27.671 2.112	99.714 0.212	10.429 0.732	7.370 0.039	6.949 0.610	3.657 0.741	30.529 2.845	47.143 13.510	0.714 0.647	0.710 0.729

pH a – arterial pH
 PaCO₂ – arterial PCO₂ (kPa)
 Pa O₂ – arterial PO₂ (kPa)
 HCO_{3a} – arterial HCO₃ (mmol.ℓ⁻¹)
 SaO₂ – arterial saturation (%)
 Hb – hemoglobin (g.dℓ⁻¹)
 pH v – coronary venous pH
 PvCO₂ – coronary venous PCO₂ (kPa)
 PvO₂ – coronary venous PO₂ (kPa)
 HCO_{3 v} – coronary venous HCO₃ (mmol.ℓ⁻¹)
 SvO₂ – coronary venous saturation (%)
 lac a – arterial lactate (mmol.ℓ⁻¹)
 lac v – venous lactate (mmol.ℓ⁻¹)

Bloodgas data

Hb remained constant throughout.

Coronary venous lactate level increased during ischemia, but returned to control levels by 10 minutes post reperfusion.

3.2.6 Bloodgas calculations

Step	CaO ₂	CvO ₂	a-vd O ₂	a-vd O ₂ %	$\dot{V}O_2$	$\dot{V}O_2/100g$	$\dot{V}O_2/100g/$ beat	CBF/100g	CBF/100g/ beat	a – v d lact
Control	15.268	5.481	9.787	64.012	3.254	8.633	0.079	90.820	0.880	0.217
	0.945	1.599	1.799	10.453	0.755	2.278	0.013	27.410	0.234	0.372
Isch	15.261	5.576	9.685	64.237	0.270	0.644	0.007	6.611	0.066	-3.914
	1.014	3.750	3.187	22.980	0.297	0.712	0.007	7.430	0.075	2.808
Rep + 10 min	15.303	12.017	3.285	21.277	2.006	5.228	0.058	169.911	1.790	-0.111
	1.097	1.366	1.497	8.845	0.695	1.697	0.026	62.418	0.694	0.531
Rep + 30 min	15.198	8.115	7.082	47.111	2.376	6.049	0.064	89.503	0.906	-0.040
	1.200	2.491	1.899	13.789	0.892	1.820	0.021	29.634	0.166	0.483
Rep + 60 min	15.143	7.447	7.696	51.355	2.530	6.585	0.068	90.720	0.891	-0.111
	1.148	2.643	2.099	15.158	0.778	1.948	0.022	29.940	0.146	0.320
Rep + 90 min	15.193	6.903	8.290	54.329	2.619	6.690	0.069	85.471	0.847	0.004
	1.184	2.022	2.322	13.491	0.753	1.459	0.019	26.492	0.186	0.489

CaO₂ – arterial oxygen content (mL.100mL⁻¹)

CvO₂ – coronary venous oxygen content (mL.100mL⁻¹)

a-v dO₂ – arterial-venous oxygen content difference (mL.100mL⁻¹)

a-v dO₂% - arterial-venous oxygen content difference as a percentage of arterial oxygen content (%)

$\dot{V}O_2$ – oxygen consumption in LAD segment (mL.min⁻¹)

$\dot{V}O_2/100g$ – oxygen consumption per 100g LAD segment tissue (mL.min⁻¹)

$\dot{V}O_2/100g/beat$ – oxygen consumption per 100g LAD segment tissue per beat (mL)

CBF/100g – coronary blood flow per 100g LAD segment tissue (mL.min⁻¹)

CBF/100g/beat – coronary blood flow per 100g LAD segment tissue per beat (mL)

a-v d lact – difference in arterial and coronary lactate (mmol.L⁻¹)

Arterial oxygen content remained constant throughout the experiment.

Oxygen consumption ($\dot{V}O_2$) and $\dot{V}O_2/100g$ LAD segment tissue decreased during ischemia and remained depressed until 60 minutes post reperfusion when values once again approached control values. $\dot{V}O_2$ and $\dot{V}O_2/100g$ LAD segment tissue were however consistently greater at all times during reperfusion, than during ischemia.

$\dot{V}O_2/100g/beat$ decreased during ischemia, remained decreased at 10 minutes after reperfusion, but had returned to control values by 30 minutes after reperfusion. Values during reperfusion were, at all times, greater than during ischemia.

CBF/100g and CBF/100g/beat in the LAD segment decreased from control values during ischemia and increased from control values at 10 minutes post reperfusion and returned to control values at 30 minutes post reperfusion where it remained for the rest of the experiment.

The difference in arterial and venous lactate levels increased during ischemia, and returned to control values at 10 minutes post reperfusion where it remained for the rest of the experiment.

3.3 Verapamil 2mg ($8\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) started 3 minutes before reperfusion and continued for 8 minutes

3.3.1 General data

Step	Tot mass	Isch mass	Vent mass	Temp	CBF	HR	MAP	Pes	Ped	+dP/dt	-dP/dt
Control	32 2.769	44.486 15.495	93.701 15.922	38.129 0.892	22.143 13.372	90.143 14.531	83.429 15.925	91.611 18.522	13.849 4.017	1247.883 175.552	1461.109 295.397
Isch	32 2.769	44.486 15.495	93.701 15.922	38.104 0.985	0.643 1.092	97.143 5.757	75.571 11.703	81.363 14.352	17.089 5.192	1027.020 259.218	1184.844 243.899
Rep + 10 min	32 2.769	44.486 15.495	93.701 15.922	37.986 0.942	43.857 9.371	97.143 11.466	73.857 6.842	80.829 8.645	17.651 6.135	1053.980 90.474	1238.577 267.099
Rep + 30 min	32 2.769	44.486 15.495	93.701 15.922	37.486 1.579	20.143 3.288	88.714 10.339	81.429 8.904	82.879 21.516	15.356 6.309	1171.329 189.674	1391.809 265.139
Rep + 60 min	32 2.769	44.486 15.495	93.701 15.922	37.414 1.596	21.714 4.112	90.000 13.844	86.000 9.092	96.854 9.100	12.597 4.086	1093.794 478.146	1454.746 267.164
Rep + 90 min	32 2.769	44.486 15.495	93.701 15.922	37.314 1.782	20.286 5.282	90.143 18.371	85.000 5.000	95.627 7.616	13.643 5.995	1176.987 212.745	1419.150 259.958

Tot mass – total animal mass (kg)

Isch mass – ischemic segment mass (g)

Vent mass – left ventricle mass (g)

Temp – temperature ($^{\circ}\text{C}$)

CBF – coronary bloodflow (mL/min)

HR – heart rate (min^{-1})

MAP – mean arterial pressure (mmHg)

+dP/dt – maximal positive value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

-dP/dt – maximal negative value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

Pes – left ventricular pressure at the end of systole (mmHg)

Ped – left ventricular pressure at the end of diastole (mmHg)

CBF decreased during ischemia compared to control values and all values during reperfusion. At 10 minutes post reperfusion CBF was higher than control values

and all values during the remainder of reperfusion. By 30 minutes post reperfusion CBF was no different from control values and remained so until the end of the experiment.

Heart rate remained unchanged during the experiment, as did MAP, LV endsystolic pressure and LV end diastolic pressure.

dP/dt also remained unchanged.

-dP/dt decreased during ischemia, but returned to control values by 10 minutes post reperfusion, where it remained.

3.3.2 Segment data

Step	LAD L max	LAD L min ₁	LAD L min ₂	LAD L _o	LAD Ees	LXLmax	LxL min ₁	LXL min ₂	LXL _o	LX Ees
Control	18.821 4.511	13.369 2.629	13.369 2.629	8.463 3.367	22.627 7.307	16.667 3.270	12.884 2.861	12.884 2.861	9.680 3.103	47.517 36.820
Isch	21.193 5.553	19.391 4.873	17.216 4.557	16.921 4.639	66.047 57.378	16.879 3.670	13.647 3.020	13.493 2.969	11.440 3.489	60.571 59.918
Rep + 10 min	20.764 5.124	17.220 4.055	16.301 3.702	14.794 4.470	29.704 12.839	16.950 3.207	13.467 2.714	13.330 2.770	11.377 2.262	48.519 34.402
Rep + 30 min	20.113 4.938	15.749 3.549	15.437 3.431	10.427 2.209	22.367 13.468	17.030 3.258	13.349 2.847	13.349 2.847	10.804 2.470	43.669 27.615
Rep + 60 min	19.643 4.688	15.130 3.385	15.043 3.379	10.774 2.452	26.987 14.784	16.747 3.146	13.193 2.927	13.193 2.927	10.944 2.302	88.181 130.951
Rep + 90 min	19.134 4.894	14.859 3.432	14.789 3.441	11.189 3.057	26.850 17.396	16.457 3.488	13.203 3.107	13.203 3.107	10.666 2.694	56.633 45.660

LAD Lmax – maximum LAD segment length (mm)

LAD Lmin₁ – minimum LAD segment length at the end of systole (mm)

LAD Lmin₂ – minimum LAD segment length (mm)

LAD L_0 – LAD segment length when left ventricular pressure is zero (mm)
LAD Ees – end systolic elastance of the LAD segment (mmHg.mm⁻¹)
LX Lmax – maximum circumflex segment length (mm)
LX Lmin₁ – minimum circumflex segment length at the end of systole (mm)
LX Lmin₂ – minimum circumflex length (mm)
LX L_0 – circumflex segment length when left ventricular pressure is zero (mm)
LX Ees – end systolic elastance of the circumflex segment (mmHg.mm⁻¹)

LAD segment

Maximum LAD segment length (LAD Lmax) increased during ischemia, remained increased at 10 minutes post reperfusion compared to control values, and returned to control values by 30 minutes post reperfusion.

LAD L_0 (unstressed LAD segment length) increased during ischemia, remained increased at 10 minutes post reperfusion, but had returned to control values by 30 minutes post reperfusion.

LAD Ees remained unchanged throughout.

LX segment

LX Lmax, LX L_0 and LX Ees remained unchanged during the experiments.

3.3.3 LAD segment calculations

Step	LADtot dL	LAD syst dL	LAD syst dL %	LAD PSS	LAD PSS %	LAD SW	LAD stat C
Control	5.453	5.453	28.114	0.000	0.000	479.679	1.429
	2.193	2.193	5.979	0.000	0.000	169.939	0.443
Isch	3.977	1.801	8.262	2.176	54.512	148.181	1.313
	1.435	1.470	5.495	1.379	26.461	121.237	0.486
Rep + 10 min	4.463	3.544	16.776	0.919	21.023	290.009	1.234
	1.708	1.776	5.512	0.710	15.434	164.047	0.290
Rep + 30 min	4.676	4.364	21.197	0.311	7.715	364.500	1.400
	1.810	2.081	6.874	0.777	18.907	203.661	0.373
Rep + 60 min	4.600	4.513	22.631	0.087	2.110	427.152	1.648
	1.626	1.671	4.992	0.231	5.583	132.127	0.526
Rep + 90 min	4.346	4.276	21.892	0.070	1.809	408.221	1.500
	1.639	1.673	3.888	0.185	4.786	169.558	0.402

LAD tot dL – total shortening of LAD segment (mm)

LAD syst dL – total systolic shortening of LAD segment (mm)

LAD syst dL% - LAD segment systolic shortening as a percentage of maximum LAD segment length (%)

LAD PSS – LAD segment postsystolic shortening (mm)

LAD PSS% -LAD segment postsystolic shortening as a percentage of total LAD segment shortening (%)

LAD SW – LAD segment stroke work (mm.mmHg)

LAD stat C – LAD segment static compliance (mm.mmHg⁻¹)

Total LAD segment shortening (LAD tot dL) decreased during ischemia and returned to control values by 10 minutes post reperfusion.

LAD segment systolic shortening (LAD syst dL) and LAD segment systolic shortening as a percentage of maximum LAD segment length (LAD syst dL%), decreased during ischemia and returned to control values by 30 minutes post

reperfusion. The value at 10 minutes post reperfusion was greater than that during ischemia, but smaller than both control, and 30 minutes post reperfusion values.

LAD segment PSS, and PSS as a percentage of total LAD segment shortening, increased during ischemia, but returned to control values by 10 minutes post reperfusion.

LAD segment stroke work decreased during ischemia, and returned to control values by 30 minutes post reperfusion.

LAD segment static compliance remained unchanged during the experiments.

3.3.4 Circumflex segment calculations

Step	LX tot dL	LX syst dL	LX syst dL%	LX PSS	LX PSS%	LX SW	LX stat C
Control	3.783	3.783	22.961	0.000	0.000	347.889	1.292
	0.815	0.815	4.141	0.000	0.000	112.936	0.431
Isch	3.386	3.231	19.030	0.154	5.714	268.471	1.082
	1.019	1.205	5.271	0.408	15.119	121.124	0.424
Rep + 10 min	3.620	3.483	20.582	0.137	3.204	276.581	1.028
	0.961	0.918	3.917	0.363	8.478	58.070	0.267
Rep + 30 min	3.681	3.681	21.791	0.000	0.000	308.536	1.209
	1.077	1.077	4.985	0.000	0.000	118.736	0.358
rep + 60 min	3.554	3.554	21.540	0.000	0.000	344.383	1.403
	1.023	1.023	5.542	0.000	0.000	99.973	0.388
rep + 90 min	3.254	3.254	19.928	0.000	0.000	309.749	1.305
	1.027	1.027	5.078	0.000	0.000	93.893	0.359

LX tot dL – LX segment total shortening (mm)

LX syst dL – LX segment total systolic shortening (mm)

LX syst dL% - LX segment systolic shortening as a percentage of maximum LX segment length (%)

LX PSS – LX segment post systolic shortening (mm)

LX PSS% - LX segment post systolic shortening as a percentage of total LX segment shortening (%)

LX SW – LX segment stroke work (mm.mmHg)

LX stat C – LX segment static compliance (mm.mmHg⁻¹)

LX tot dL, LX syst dL, LX syst dL%, LX PSS, LX PSS% and LX stat C remained constant throughout the experiment.

3.3.5 Bloodgas data

Step	pH a	PaCO ₂	PaO ₂	HCO _{3a}	SaO ₂	Hb	pH v	PvCO ₂	PvO ₂	HCO _{3 v}	SvO ₂	lac a	lac v
Control	7.436 0.053	5.853 1.040	32.229 19.333	29.200 2.873	99.286 1.129	10.500 1.258	7.360 0.032	7.553 0.959	3.271 0.886	31.986 2.572	39.814 15.880	0.586 0.832	0.586 0.691
Isch	7.456 0.047	5.551 0.759	37.129 18.776	28.271 2.854	99.757 0.237	10.500 1.258	7.304 0.078	8.377 1.627	3.200 0.860	31.014 2.556	35.486 14.783	0.786 0.946	1.857 1.024
Rep + 10 min	7.429 0.054	5.617 0.966	33.071 22.206	27.814 3.011	98.957 1.191	10.500 1.258	7.383 0.086	6.479 1.414	6.443 0.608	28.471 2.370	81.400 6.627	0.729 0.877	1.100 0.847
Rep + 30 min	7.423 0.060	5.649 0.917	34.686 22.651	27.614 2.896	99.000 1.420	10.500 1.258	7.397 0.036	6.366 0.773	4.443 0.852	29.214 2.455	61.357 13.268	0.771 0.739	0.614 0.722
Rep + 60 min	7.420 0.062	5.703 0.994	34.086 22.833	27.543 2.617	98.657 2.857	10.500 1.258	7.369 0.045	6.931 0.758	3.914 0.838	29.800 1.169	51.200 14.705	0.871 0.655	0.729 0.725
Rep + 90 min	7.429 0.053	5.596 0.920	34.714 22.035	27.557 2.578	98.643 3.027	10.643 1.376	7.367 0.046	7.016 0.955	3.586 0.669	29.986 1.874	46.214 13.636	0.686 0.615	0.786 0.604

pH a – arterial pH

PaCO₂ – arterial PCO₂ (kPa)

Pa O₂ – arterial PO₂ (kPa)

HCO_{3a} – arterial HCO₃ (mmol.ℓ⁻¹)

SaO₂ – arterial saturation (%)

Hb – hemoglobin (g.dℓ⁻¹)

pH v – coronary venous pH

PvCO₂ – coronary venous PCO₂ (kPa)

PvO₂ – coronary venous PO₂ (kPa)

HCO_{3 v} – coronary venous HCO₃ (mmol.ℓ⁻¹)

SvO₂ – coronary venous saturation (%)

lac a – arterial lactate (mmol.ℓ⁻¹)

lac v – coronary venous lactate (mmol.ℓ⁻¹)

Hb and coronary venous lactate remained unchanged during the experiments.

3.3.6 Bloodgas calculations

Step	CaO ₂	CvO ₂	a-v dO ₂	a-v dO ₂ %	$\dot{V}O_2$	$\dot{V}O_2/100g$	$\dot{V}O_2/100g/beat$	CBF/100g	CBF/100g/beat	a-v d lact
Control	15.237	5.994	9.243	61.474	2.006	4.210	0.048	45.961	0.533	0.000
	1.845	2.480	1.790	14.915	1.310	1.681	0.020	15.655	0.231	0.462
Isch	15.423	5.440	9.983	66.051	0.063	0.112	0.001	1.171	0.012	-1.071
	1.932	2.648	1.138	13.584	0.111	0.156	0.002	1.602	0.016	1.409
Rep + 10 min	15.212	12.075	3.136	20.891	1.355	3.250	0.033	105.072	1.072	-0.371
	1.981	2.089	0.730	5.456	0.320	1.017	0.009	29.484	0.221	0.741
Rep + 30 min	15.249	9.220	6.028	40.614	1.241	3.002	0.034	49.583	0.560	0.157
	1.956	2.823	1.325	12.338	0.431	1.291	0.014	17.199	0.188	0.351
Rep + 60 min	15.185	7.713	7.472	50.323	1.620	3.738	0.042	52.310	0.588	0.143
	2.031	2.701	1.357	12.858	0.437	0.503	0.005	15.653	0.189	0.326
Rep + 90 min	15.393	7.035	8.358	55.115	1.731	3.935	0.044	47.309	0.538	-0.100
	2.132	2.404	1.584	12.521	0.682	1.073	0.012	9.910	0.135	0.346

CaO₂ – arterial oxygen content (mL.100mL⁻¹)

CvO₂ – coronary venous oxygen content (mL.100mL⁻¹)

a-v dO₂ – arterial-venous oxygen content difference (mL.100mL⁻¹)

a-v dO₂% - arterial-venous oxygen content difference as a percentage of arterial oxygen content (%)

$\dot{V}O_2$ – oxygen consumption in LAD segment (mL.min⁻¹)

$\dot{V}O_2/100g$ – oxygen consumption per 100g LAD segment tissue (mL.min⁻¹)

$\dot{V}O_2/100g/beat$ – oxygen consumption per 100g LAD segment tissue per beat (mL)

CBF/100g – coronary blood flow per 100g LAD segment tissue (mL.min⁻¹)

CBF/100g/beat – coronary blood flow per 100g LAD segment tissue per beat (mL)

a-v d lact – difference in arterial and coronary venous lactate (mmol.L⁻¹)

Arterial oxygen content remained unchanged throughout the experiment.

VO_2 , $VO_2/100g$ and $VO_2/100g/beat$ in the LAD segment decreased during ischemia, but had returned to control values by 10 minutes post reperfusion, and remained so for the rest of the experiment.

CBF/100g decreased during ischemia, and increased at 10 minutes post reperfusion to values higher than both control values, and values at 30 minutes post reperfusion. By 30 minutes post reperfusion CBF/100g was again the same as controls.

CBF/100g/beat was increased at 10 minutes post reperfusion compared to ischemic values, and compared to values during the rest of reperfusion.

The difference in arterial and coronary venous lactate remained unchanged throughout the experiment.

3.4 Saline infusion started 3 minutes before reperfusion and continued for 8 minutes.

3.4.1 General data

Step	Tot mass	Isch mass	Vent mass	Temp	CBF	HR	MAP	Pes	Ped	+dP/dt	-dP/dt
Control	30.857 5.551	45.356 9.549	98.157 25.450	38.100 0.737	26.000 5.508	93.000 10.231	80.286 16.997	87.484 19.977	7.904 2.812	1019.714 421.049	1402.260 324.548
Isch	30.857 5.551	45.356 9.549	98.157 25.450	37.871 0.716	1.643 2.774	98.143 13.459	71.143 23.611	74.924 27.038	11.849 3.832	1113.129 363.023	1167.880 410.284
Rep + 10 min	30.857 5.551	45.356 9.549	98.157 25.450	37.914 0.527	45.857 21.874	98.714 18.795	77.857 14.311	83.147 17.231	13.060 3.230	1071.649 204.832	1319.491 273.762
Rep + 30 min	30.857 5.551	45.356 9.549	98.157 25.450	37.857 0.461	26.000 10.017	94.571 9.414	80.714 14.044	88.010 15.185	13.163 3.441	1139.914 169.362	1368.017 213.282
Rep + 60 min	30.857 5.551	45.356 9.549	98.157 25.450	37.871 0.468	24.714 8.789	91.429 11.559	79.571 16.682	87.769 18.319	12.791 3.416	1022.340 239.886	1277.316 259.062
Rep + 90 min	30.857 5.551	45.356 9.549	98.157 25.450	37.643 0.635	21.571 8.810	98.143 8.971	74.857 19.970	80.893 23.305	12.277 1.592	1001.600 267.726	1194.369 343.227

Tot mass – total animal mass (kg)

Isch mass – ischemic segment mass (g)

Vent mass – left ventricle mass (g)

Temp – temperature (°C)

CBF – coronary bloodflow (ml/min)

HR – heart rate (min⁻¹)

MAP – mean arterial pressure (mmHg)

+dP/dt – maximal positive value of the instantaneous first derivative of left ventricular pressure (mmHg.sec⁻¹)

-dP/dt – maximal negative value of the instantaneous first derivative of left ventricular pressure (mmHg.sec⁻¹)

Pes – left ventricular pressure at the end of systole (mmHg)

Ped – left ventricular pressure at the end of diastole (mmHg)

CBF decreased during ischemia, and was increased at 10 minutes post reperfusion compared to 90 minutes post reperfusion. By 30 minutes post

reperfusion CBF had returned to control values, and would remain so until the end of the experiment.

Heart rate, MAP, LV end systolic pressure and dP/dt remained unchanged during the experiments.

$-dP/dt$ decreased during ischemia then recovered to control values, only to decrease again compared to control values at 90 minutes post reperfusion.

LV end diastolic pressure increased during ischemia, and remained increased compared to control values, for the duration of the experiment

3.4.2 Segment data

Step	LAD L _{max}	LAD L _{min₁}	LAD L _{min₂}	LAD L _o	LAD Ees	LXL _{max}	LxL _{min₁}	LXL _{min₂}	LXL _o	LX Ees
Control	18.039 3.363	13.184 3.031	12.953 2.759	8.789 4.644	30.083 19.982	16.130 3.867	12.149 2.232	12.144 2.223	9.253 1.902	44.936 31.633
Isch	19.811 3.536	18.781 3.623	16.681 3.031	16.219 4.144	52.120 27.714	16.721 4.095	12.410 2.748	12.411 2.749	9.820 3.033	36.656 12.035
Rep + 10 min	19.461 3.746	17.239 4.024	15.276 3.430	11.509 3.531	19.399 5.431	16.646 4.231	12.774 2.615	12.776 2.616	10.163 2.447	35.801 12.679
Rep + 30 min	18.983 3.864	16.744 4.247	15.051 3.417	9.493 5.465	17.114 10.524	16.383 4.453	12.466 3.128	12.466 3.128	8.851 3.969	31.491 16.895
Rep + 60 min	18.496 3.998	16.114 4.068	14.921 3.420	7.815 6.331	15.859 11.629	16.189 4.352	12.296 2.971	12.296 2.971	8.669 3.286	27.431 6.711
Rep + 90 min	18.297 3.656	15.663 4.110	14.829 3.580	7.765 7.194	15.574 10.313	15.900 3.788	12.779 3.745	12.779 3.745	9.087 3.730	25.077 6.097

LAD L_{max} – maximum LAD segment length (mm)

LAD L_{min₁} – minimum LAD segment length at the end of systole (mm)

LAD L_{min₂} – minimum LAD segment length (mm)

LAD L_o – LAD segment length when left ventricular pressure is zero (mm)

LAD Ees – end systolic elastance of the LAD segment (mmHg.mm⁻¹)

LX L_{max} – maximum circumflex segment length (mm)

LX L_{min₁} – minimum circumflex segment length at the end of systole (mm)

LX L_{min₂} – minimum circumflex length (mm)

LX L_o – circumflex segment length when left ventricular pressure is zero (mm)

LX Ees – end systolic elastance of the circumflex segment (mmHg.mm⁻¹)

LAD segment

Maximum LAD segment length (LAD L_{max}) increased during ischemia, and only decreased again at 60 minutes post reperfusion.

LAD L_o (unstressed LAD segment length) increased during ischemia and returned to control values by 10 minutes post reperfusion.

LAD Ees decreased from 60 minutes post reperfusion onwards, compared to control values.

LX segment

LX Lmax, LX Lo, LX Ees remained unchanged during the experiments.

3.4.3 LAD segment calculations

Step	LADtot dL	LAD syst dL	LAD syst dL %	LAD PSS	LAD PSS %	LAD SW	LAD stat C
Control	5.086	4.854	27.220	0.231	4.923	412.370	2.459
	1.115	1.230	6.311	0.397	8.491	86.636	0.800
Isch	3.130	1.030	5.381	2.100	67.218	76.970	1.876
	1.089	0.544	3.433	0.890	13.421	48.869	0.866
Rep + 10 min	4.186	2.223	11.981	1.963	47.907	184.148	1.516
	0.883	1.103	7.529	0.886	18.637	102.775	0.255
Rep + 30 min	3.931	2.239	12.606	1.693	42.464	193.511	1.512
	0.790	0.849	7.214	0.937	20.289	67.160	0.456
Rep + 60 min	3.574	2.381	13.517	1.193	32.313	206.736	1.604
	0.746	0.618	5.279	0.732	18.205	55.192	0.789
Rep + 90 min	3.469	2.634	15.173	0.834	27.162	210.288	1.507
	0.854	1.281	7.690	0.669	26.174	92.933	0.347

LAD tot dL – total shortening of LAD segment (mm)

LAD syst dL – total systolic shortening of LAD segment (mm)

LAD syst dL% - LAD segment systolic shortening as a percentage of maximum LAD segment length (%)

LAD PSS – LAD segment postsystolic shortening (mm)

LAD PSS% - LAD segment postsystolic shortening as a percentage of total LAD segment shortening (%)

LAD SW – LAD segment stroke work (mm.mmHg)

LAD stat C – LAD segment static compliance (mm.mmHg⁻¹)

Total LAD segment shortening decreased during ischemia, and despite an initial recovery to control values at 10 minutes post reperfusion, it decreased again by 30 minutes post reperfusion and remained decreased at the end of the experiment at 90 minutes post reperfusion.

LAD segment systolic shortening (LAD syst dL) and LAD segment systolic shortening as a percentage of maximum LAD segment length (LAD syst dL%) decreased during ischemia, and even though the post ischemic values were higher than the ischemic values, control values has not been reached by the end of the experiment at 90 minutes post reperfusion.

LAD segment PSS increased during ischemia and remained increased until 90 minutes post reperfusion when control values were once again approached.

LAD segment PSS as a percentage of total LAD segment shortening increased during ischemia, and although from 30 minutes post reperfusion the values were lower than ischemic values, control values had not been reached by the end of the experiment at 90 minutes post reperfusion.

LAD segment stroke work decreased during ischemia, and although post reperfusion values all increased compared to ischemic values, control values had not been reached by the end of the experiment at 90 minutes post reperfusion.

LAD segment static compliance was decreased at 10 minutes post reperfusion, and remained decreased at the end of the experiment at 90 minutes post reperfusion.

3.4.4 Circumflex segment calculations

Step	LX tot dL	LX syst dL	LX syst dL%	LX PSS	LX PSS%	LX SW	LX stat C
Control	3.986	3.981	23.939	0.004	0.054	326.055	2.139
	1.816	1.805	4.786	0.011	0.144	82.530	0.509
Isch	4.310	4.311	25.508	-0.001	-0.032	315.511	1.509
	1.427	1.427	2.416	0.004	0.086	112.828	0.441
Rep + 10 min	3.870	3.871	22.517	-0.001	-0.035	302.750	1.277
	1.820	1.821	5.621	0.004	0.092	95.152	0.134
Rep + 30 min	3.917	3.917	23.607	0.000	0.000	332.533	1.277
	1.558	1.558	4.911	0.000	0.000	102.410	0.310
Rep + 60 min	3.893	3.893	23.654	0.000	0.000	329.836	1.353
	1.567	1.567	4.171	0.000	0.000	105.656	0.493
Rep + 90 min	3.121	3.121	20.192	0.000	0.000	249.070	1.300
	0.976	0.976	5.862	0.000	0.000	84.613	0.292

LX tot dL – LX segment total shortening (mm)

LX syst dL – LX segment total systolic shortening (mm)

LX syst dL% - LX segment systolic shortening as a percentage of maximum LX segment length (%)

LX PSS – LX segment post systolic shortening (mm)

LX PSS% - LX segment post systolic shortening as a percentage of total LX segment shortening (%)

LX SW – LX segment stroke work (mm.mmHg)

LX stat C – LX segment static compliance (mm.mmHg⁻¹)

LX tot dL, LX syst dL, LX syst dL%, LXPSS, LX PSS%, and LX stat C remained constant throughout the experiment.

3.4.5 Bloodgas data

Step	pH a	PaCO ₂	PaO ₂	HCO ₃ a	SaO ₂	Hb	pH v	PvCO ₂	PvO ₂	HCO ₃ v	SvO ₂	lac a	lac v
Control	7.384 0.021	6.281 0.985	36.000 18.055	28.586 2.742	98.471 3.400	9.857 0.802	7.300 0.033	8.421 0.901	3.171 0.509	34.014 4.642	35.014 8.083	0.357 0.454	0.243 0.378
Isch	7.386 0.033	6.034 0.591	37.171 23.311	28.729 1.845	99.786 0.195	9.857 0.802	7.209 0.031	10.594 1.548	2.943 0.458	31.229 2.653	25.629 6.650	0.457 0.643	3.329 1.935
Rep + 10 min	7.379 0.047	6.201 0.854	36.743 22.486	28.243 1.791	99.229 1.365	9.857 0.802	7.363 0.052	6.959 1.048	5.371 1.855	29.800 2.085	66.714 20.690	0.800 0.723	0.743 0.685
Rep + 30 min	7.390 0.038	5.920 0.576	38.843 19.833	27.943 1.868	99.729 0.325	9.643 0.556	7.334 0.036	7.173 0.705	4.029 0.907	29.857 1.585	52.014 15.504	0.829 0.553	0.800 0.493
Rep + 60 min	7.399 0.038	5.734 0.648	39.129 19.653	27.857 1.389	99.671 0.571	9.643 0.556	7.337 0.035	7.241 0.768	3.671 0.716	30.386 2.237	46.371 12.295	0.900 0.632	0.843 0.553
Rep + 90 min	7.393 0.043	5.723 0.655	38.671 18.032	27.343 1.758	99.643 0.645	9.714 0.488	7.347 0.060	7.269 0.916	3.571 0.898	30.086 2.900	44.186 14.972	0.857 0.602	2.200 3.335

pH a – arterial pH

PaCO₂ – arterial PCO₂ (kPa)

Pa O₂ – arterial PO₂ (kPa)

HCO₃a – arterial HCO₃ (mmol.ℓ⁻¹)

SaO₂ – arterial saturation (%)

Hb – hemoglobin (g.dℓ⁻¹)

pH v – coronary venous pH

PvCO₂ – coronary venous PCO₂ (kPa)

PvO₂ – coronary venous PO₂ (kPa)

HCO₃ v – coronary venous HCO₃ (mmol.ℓ⁻¹)

SvO₂ – coronary venous saturation (%)

lac a – arterial lactate (mmol.ℓ⁻¹)

lac v – coronary venous lactate (mmol.ℓ⁻¹)

Hb remained unchanged during the experiments. Coronary venous lactate increased during ischemia, but returned to control values by 10 minutes post reperfusion.

3.4.6 Bloodgas calculations

Step	CaO ₂	CvO ₂	a-v dO ₂	a-v dO ₂ %	$\dot{V}O_2$	$\dot{V}O_2/100g$	$\dot{V}O_2/100g/$ beat	CBF/100g	CBF/100g/ beat	a-v d lact
Control	14.336	4.895	9.441	65.972	2.418	5.604	0.060	60.654	0.646	0.114
	1.514	1.377	1.376	7.576	0.446	1.760	0.017	19.920	0.188	0.659
Isch	14.537	3.599	10.939	75.454	0.190	0.418	0.004	3.600	0.036	-2.871
	1.484	1.000	1.067	5.693	0.337	0.756	0.008	6.171	0.065	1.391
Rep + 10 min	14.457	9.362	5.095	36.189	2.010	4.872	0.047	106.490	1.063	0.057
	1.607	3.236	2.273	18.548	0.714	3.057	0.019	56.119	0.524	0.439
Rep + 30 min	14.271	7.115	7.157	50.569	1.780	4.121	0.043	60.407	0.629	0.029
	1.108	2.285	1.789	14.331	0.637	1.583	0.014	25.309	0.250	0.454
Rep + 60 min	14.272	6.325	7.947	55.887	1.877	4.351	0.047	56.860	0.620	0.057
	1.162	1.793	1.500	11.132	0.489	1.378	0.015	21.000	0.232	0.476
Rep + 90 min	14.355	6.117	8.237	57.993	1.785	4.198	0.043	50.265	0.519	-1.343
	1.007	2.317	1.585	13.540	0.697	1.747	0.018	21.432	0.235	2.899

CaO₂ – arterial oxygen content (mL.100mL⁻¹)

CvO₂ – coronary venous oxygen content (mL.100mL⁻¹)

a-v dO₂ – arterial-venous oxygen content difference (mL.100mL⁻¹)

a-v dO₂% - arterial-venous oxygen content difference as a percentage of arterial oxygen content (%)

$\dot{V}O_2$ – oxygen consumption in LAD segment (mL.min⁻¹)

$\dot{V}O_2/100g$ – oxygen consumption per 100g LAD segment tissue (mL.min⁻¹)

$\dot{V}O_2/100g/beat$ – oxygen consumption per 100g LAD segment tissue per beat (mL)

CBF/100g – coronary blood flow per 100g LAD segment tissue (mL.min⁻¹)

CBF/100g/beat – coronary blood flow per 100g LAD segment tissue per beat (mL)

a-v d lact – difference in arterial and coronary venous lactate (mmol.L⁻¹)

Arterial oxygen content remained unchanged throughout the experiment.

VO_2 , $VO_2/100g$ and $VO_2/100g/beat$ in the LAD segment decreased during ischemia, but had returned to control values by 10 minutes post reperfusion, and remained so for the rest of the experiment.

CBF/100g in the LAD segment decreased during ischemia, and increased at 10 minutes post reperfusion to values higher than both control values, and values during the remainder of the reperfusion. By 30 minutes post reperfusion CBF/100g was the same as control values, and remained so.

CBF/100g/beat in the LAD segment decreased during ischemia and returned to control values by 10 minutes post reperfusion, and remained so.

The difference in arterial and coronary venous lactate increased during ischemia, and returned to control values by 10 minutes post reperfusion.

3.5 Verapamil 2mg ($8\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) started at the onset of ischemia and continued for 8 minutes

3.5.1 General data

Step	Tot mass	Isch mass	Vent mass	Temp	CBF	HR	MAP	Ped	Pes	+dP/dt	-dP/dt
Control	28.833 2.639	36.098 5.759	91.103 10.516	38.517 0.804	25.500 11.879	99.000 12.977	67.667 13.648	6.742 1.858	72.603 15.174	1196.403 297.971	1253.757 132.334
Isch	28.833 2.639	36.098 5.759	91.103 10.516	38.350 0.841	0.750 1.592	98.833 15.420	60.500 9.138	9.572 1.512	63.883 10.792	1059.982 190.441	1077.260 165.482
Rep + 10 min	28.833 2.639	36.098 5.759	91.103 10.516	38.317 0.997	49.000 11.730	99.500 11.041	63.500 8.019	9.563 2.301	68.105 7.960	1085.090 188.310	1181.925 146.940
Rep + 30 min	28.833 2.639	36.098 5.759	91.103 10.516	38.200 0.906	29.833 8.085	99.667 10.838	72.333 8.847	8.590 2.393	80.410 9.692	1253.888 311.463	1334.568 168.914
Rep + 60 min	28.833 2.639	36.098 5.759	91.103 10.516	38.150 0.963	33.667 10.727	107.667 16.741	78.167 11.125	7.700 2.944	85.718 11.703	1084.253 549.734	1366.203 241.468
Rep + 90 min	28.833 2.639	36.098 5.759	91.103 10.516	38.033 0.850	32.333 11.570	100.167 12.608	77.167 22.266	8.830 2.998	86.825 24.106	1228.285 381.280	1325.587 396.550

Tot mass – total animal mass (kg)

Isch mass – ischemic segment mass (g)

Vent mass – left ventricle mass (g)

Temp – temperature ($^{\circ}\text{C}$)

CBF – coronary bloodflow (mL/min)

HR – heart rate (min^{-1})

MAP – mean arterial pressure (mmHg)

+dP/dt – maximal positive value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

-dP/dt – maximal negative value of the instantaneous first derivative of left ventricular pressure ($\text{mmHg}\cdot\text{sec}^{-1}$)

Pes – left ventricular pressure at the end of systole (mmHg)

Ped – left ventricular pressure at the end of diastole (mmHg)

CBF decreased during ischemia and increased at 10 minutes post reperfusion

compared to both control, and other post reperfusion values. By 30 minutes

post reperfusion CBF had returned to control values, and remained so until the end of the experiment.

Heart rate remained unchanged during the experiment.

MAP and LV end systolic pressure was higher at 60 and 90 minutes post reperfusion than during ischemia, but no different at any time from control values. LVEDP remained unchanged throughout.

dP/dt remained unchanged during the experiment.

-dP/dt was higher at 30 and 60 minutes post reperfusion, than during ischemia, but no different at any time from control values.

3.5.2 Segment data

Step	LAD L max	LAD L min ₁	LAD L min ₂	LAD L _o	LAD Ees	LXLmax	LxL min ₁	LXL min ₂	LXL _o	LX Ees
Control	16.958 2.858	12.524 2.958	12.524 2.958	10.458 2.236	37.360 13.454	15.418 2.463	11.675 2.000	11.675 2.000	9.065 1.870	41.700 19.545
Isch	18.723 3.410	18.346 3.757	16.042 3.007	15.032 3.813	51.765 28.763	16.338 2.569	12.528 2.206	12.528 2.206	10.565 2.401	40.433 12.572
Rep + 10 min	17.967 3.476	14.577 3.768	14.102 3.483	11.733 3.183	30.568 10.352	16.398 2.502	12.538 2.015	12.538 2.015	10.747 2.126	45.558 15.729
Rep + 30 min	17.170 3.395	13.627 4.202	13.108 3.411	10.588 2.634	36.085 15.499	15.980 2.460	12.040 2.023	12.040 2.023	10.017 1.682	44.637 12.607
Rep + 60 min	16.580 2.928	13.177 3.275	12.992 3.062	9.892 2.673	31.487 11.569	15.667 2.575	12.085 2.271	12.085 2.271	9.590 1.744	40.487 11.261
Rep + 90 min	16.325 2.689	12.840 2.675	12.737 2.626	9.583 3.172	34.858 17.945	15.388 2.712	12.042 2.416	12.042 2.416	8.577 3.434	45.197 22.661

LAD Lmax – maximum LAD segment length (mm)

LAD Lmin₁ – minimum LAD segment length at the end of systole (mm)

LAD L_{min_2} – minimum LAD segment length (mm)

LAD L_o – LAD segment length when left ventricular pressure is zero (mm)

LAD Ees – end systolic elastance of the LAD segment ($\text{mmHg} \cdot \text{mm}^{-1}$)

LX L_{max} – maximum circumflex segment length (mm)

LX L_{min_1} – minimum circumflex segment length at the end of systole (mm)

LX L_{min_2} – minimum circumflex length (mm)

LX L_o – circumflex segment length when left ventricular pressure is zero (mm)

LX Ees – end systolic elastance of the circumflex segment ($\text{mmHg} \cdot \text{mm}^{-1}$)

LAD segment

Maximum LAD segment length (LAD L_{max}) increased during ischemia, but had returned to control values by 10 minutes post reperfusion.

LAD L_o (unstressed LAD segment length) increased during ischemia, but had returned to control values by 10 minutes post reperfusion.

LAD Ees remained unchanged throughout.

LX segment

LX L_{max} increased during ischemia, remained increased at 10 minutes post reperfusion, but had returned to control values by 30 minutes post reperfusion (the values at 30 and 60 minutes post reperfusion were no different from those at 10 minutes post reperfusion, during ischemia, or control values).

LX L_o and LX Ees remained unchanged during the experiment.

3.5.3 LAD segment calculations

Step	LADtot dL	LAD syst dL	LAD syst dL %	LAD PSS	LAD PSS %	LAD SW	LAD stat C
Control	4.152	3.975	23.786	0.000	0.000	290.080	2.647
	0.894	1.097	6.730	0.000	0.000	109.062	0.738
Isch	3.135	1.145	6.588	2.304	68.698	72.605	1.969
	0.695	0.566	3.907	0.841	20.304	34.228	0.276
Rep + 10 min	3.865	3.390	19.481	0.475	12.230	235.253	1.956
	0.776	0.873	5.891	0.543	12.406	85.872	0.566
Rep + 30 min	4.062	3.543	21.679	0.518	13.222	290.236	2.143
	0.967	1.234	8.045	0.831	23.362	122.778	0.780
Rep + 60 min	3.588	3.403	21.124	0.185	5.355	299.295	2.463
	0.986	0.939	6.033	0.287	8.983	121.489	1.121
Rep + 90 min	3.588	3.485	21.689	0.103	2.026	316.016	2.007
	0.909	0.718	4.444	0.253	4.963	150.913	0.695

LAD tot dL – total shortening of LAD segment (mm)

LAD syst dL – total systolic shortening of LAD segment (mm)

LAD syst dL% - LAD segment systolic shortening as a percentage of maximum LAD segment length (%)

LAD PSS – LAD segment postsystolic shortening (mm)

LAD PSS% - LAD segment postsystolic shortening as a percentage of total LAD segment shortening (%)

LAD SW – LAD segment stroke work (mm.mmHg)

LAD stat C – LAD segment static compliance (mm.mmHg⁻¹)

Total LAD segment shortening (LAD tot dL) decreased during ischemia, and returned to control values by 10 minutes post reperfusion.

LAD segment systolic shortening (LAD syst dL) and LAD segment systolic shortening as a percentage of maximum LAD segment length (LAD syst dL%),

decreased during ischemia and returned to control values by 10 minutes post reperfusion.

LAD segment PSS and PSS as a percentage of total LAD segment length increased during ischemia, but had returned to control values by 10 minutes after reperfusion.

LAD segment stroke work decreased during ischemia, and returned to control values by 10 minutes post reperfusion.

LAD segment static compliance remained unchanged during the experiments.

3.5.4 Circumflex segment calculations

Step	LX tot dL	LX syst dL	LX syst dL%	LX PSS	LX PSS%	LX SW	LX stat C
Control	3.743	3.743	24.406	0.000	0.000	277.139	2.383
	0.529	0.529	1.976	0.000	0.000	100.238	0.549
Isch	3.810	3.810	23.432	0.000	0.000	251.335	1.723
	0.969	0.969	4.550	0.000	0.000	116.399	0.250
Rep + 10 min	3.860	3.860	23.555	0.000	0.000	263.430	1.750
	0.974	0.974	4.181	0.000	0.000	76.280	0.233
Rep + 30 min	3.940	3.940	24.682	0.000	0.000	323.056	1.979
	1.155	1.155	5.426	0.000	0.000	132.667	0.568
Rep + 60 min	3.582	3.582	23.026	0.000	0.000	313.157	2.286
	1.021	1.021	5.346	0.000	0.000	132.979	0.898
Rep + 90 min	3.347	3.347	21.962	0.000	0.000	300.823	1.861
	0.922	0.922	5.054	0.000	0.000	167.263	0.491

LX tot dL – LX segment total shortening (mm)

LX syst dL – LX segment total systolic shortening (mm)

LX syst dL% - LX segment systolic shortening as a percentage of maximum LX segment length (%)

LX PSS - LX segment post systolic shortening (mm)

LX PSS% - LX segment post systolic shortening as a percentage of total LX segment shortening (%)

LX SW - LX segment stroke work (mm.mmHg)

LX stat C - LX segment static compliance (mm.mmHg⁻¹)

LX tot dL; LX syst dL; LX syst dL%; LX PSS; LX PSS% and LX stat C remained constant throughout the experiment.

3.5.5 Bloodgas data

Step	pH a	PaCO ₂	PaO ₂	HCO ₃ a	SaO ₂	Hb	pH v	PvCO ₂	PvO ₂	HCO ₃ v	SvO ₂	lac a	lac v
Control	7.415 0.027	5.700 0.507	35.183 10.303	32.617 4.552	99.817 0.354	10.750 1.173	7.352 0.030	7.428 0.796	3.267 0.543	35.700 4.049	42.367 12.425	0.917 0.765	0.933 0.720
Isch	7.422 0.028	5.890 0.293	34.767 12.472	32.850 6.305	99.500 1.128	10.750 1.173	7.297 0.063	8.148 0.743	3.233 0.686	32.800 4.912	37.333 8.878	1.000 0.797	2.117 1.522
rep + 10 min	7.418 0.039	6.003 0.539	32.467 15.183	31.717 3.035	99.217 1.306	10.750 1.173	7.348 0.037	6.647 0.617	5.950 0.948	33.033 5.937	79.500 6.452	0.783 0.786	1.450 1.009
Rep + 30 min	7.408 0.032	6.008 0.592	36.550 12.881	32.933 6.105	99.650 0.715	10.750 1.173	7.345 0.024	6.857 0.533	4.733 1.513	33.117 5.038	64.117 13.216	0.833 0.843	1.400 0.738
Rep + 60 min	7.413 0.027	5.810 0.608	36.183 11.855	30.867 2.335	99.767 0.432	10.750 1.173	7.330 0.032	7.122 0.865	4.217 0.337	33.900 5.429	59.150 9.249	0.900 0.654	1.050 0.838
Rep + 90 min	7.410 0.023	5.880 0.545	36.550 11.631	32.233 3.962	99.767 0.432	10.750 1.173	7.340 0.039	6.940 0.885	4.067 0.728	33.683 7.189	57.000 12.561	0.767 0.539	1.050 0.829

pH a – arterial pH

PaCO₂ – arterial PCO₂ (kPa)

Pa O₂ – arterial PO₂ (kPa)

HCO₃a – arterial HCO₃ (mmol.ℓ⁻¹)

SaO₂ – arterial saturation (%)

Hb – hemoglobin (g.dℓ⁻¹)

pH v – coronary venous pH

PvCO₂ – coronary venous PCO₂ (kPa)

PvO₂ – coronary venous PO₂ (kPa)

HCO₃ v – coronary venous HCO₃ (mmol.ℓ⁻¹)

SvO₂ – coronary venous saturation (%)

lac a – arterial lactate (mmol.ℓ⁻¹)

lac v – coronary venous lactate (mmol.l^{-1})

Hb remained constant throughout. Coronary venous lactate increased on ischemia, but had returned to control values by 10 minutes post reperfusion.

3.5.6 Bloodgas calculations

Step	CaO ₂	CvO ₂	a-v dO ₂	a-v dO ₂ %	$\dot{V}O_2$	$\dot{V}O_2/100g$	$\dot{V}O_2/100g/\text{beat}$	CBF/100g	CBF/100g/beat	a-v d lact
Control	15.730	6.419	9.311	59.355	2.327	6.273	0.064	68.864	0.693	-0.017
	1.492	2.140	1.986	11.678	1.110	2.645	0.028	28.271	0.253	0.479
Isch	15.665	5.625	10.040	64.113	0.092	0.246	0.002	2.001	0.018	-1.117
	1.351	1.375	1.503	7.857	0.203	0.534	0.005	4.196	0.038	1.332
Rep + 10 min	15.580	12.032	3.548	22.802	1.660	4.771	0.049	135.796	1.382	-0.667
	1.553	1.728	1.160	7.649	0.380	1.514	0.020	23.692	0.310	0.554
Rep + 30 min	15.733	9.633	6.100	38.522	1.801	5.070	0.051	82.191	0.825	-0.567
	1.386	1.881	2.136	12.712	0.780	2.032	0.019	16.397	0.130	0.631
Rep + 60 min	15.745	8.940	6.805	43.378	2.272	6.208	0.057	92.585	0.865	-0.150
	1.421	1.768	1.364	8.379	0.760	1.574	0.007	23.455	0.184	0.647
Rep + 90 min	15.754	8.658	7.095	45.361	2.195	6.112	0.061	88.580	0.875	-0.283
	1.431	2.283	1.721	12.114	0.687	1.686	0.014	26.299	0.176	0.665

CaO₂ – arterial oxygen content (ml.100ml^{-1})

CvO₂ – coronary venous oxygen content (ml.100ml^{-1})

a-v dO₂ – arterial-venous oxygen content difference (ml.100ml^{-1})

a-v dO₂% - arterial-venous oxygen content difference as a percentage of arterial oxygen content (%)

$\dot{V}O_2$ – oxygen consumption in LAD segment (ml.min^{-1})

$\dot{V}O_2/100g$ – oxygen consumption per 100g LAD segment tissue (ml.min^{-1})

$\dot{V}O_2/100g/\text{beat}$ – oxygen consumption per 100g LAD segment tissue per beat (ml)

CBF/100g – coronary blood flow per 100g LAD segment tissue (ml.min^{-1})

CBF/100g/beat – coronary blood flow per 100g LAD segment tissue per beat (ml)

a-v d lact – difference in arterial and coronary venous lactate (mmol.l^{-1})

Arterial oxygen content remained unchanged throughout the experiment.

VO_2 , $VO_2/100g$ and $VO_2/100g/beat$ in the LAD segment decreased during ischemia, but had returned to control values by 10 minutes post reperfusion, and remained so for the rest of the experiment.

$CBF/100g$ and $CBF/100g/beat$ in the LAD segment decreased during ischemia, and increased at 10 minutes post reperfusion to values higher than both control values, and values during the remainder of reperfusion. By 30 minutes post reperfusion, $CBF/100g$ and $CBF/100g/beat$ was the same as control values and remained so.

The difference in arterial and coronary venous lactate remained unchanged throughout the experiment.

3.6 Saline infusion started at the onset of ischemia and continued for 8 minutes.

3.6.1 General data

Step	Tot mass	Isch mass	Vent mass	Temp	CBF	HR	MAP	Pes	Ped	+dP/dt	-dP/dt
Control	30.500 2.665	26.860 5.060	90.048 9.006	38.133 0.572	24.333 3.882	108.833 10.962	71.000 5.831	74.908 7.159	9.195 3.625	1140.187 99.410	1269.550 133.756
Isch	30.500 2.665	26.860 5.060	90.048 9.006	38.060 0.677	1.480 3.086	106.200 6.301	66.800 18.674	72.688 20.991	15.614 4.064	1116.872 356.585	1107.486 334.560
Rep + 10 min	30.500 2.665	26.860 5.060	90.048 9.006	38.133 0.572	41.333 18.239	109.000 16.297	77.500 6.535	82.935 11.177	11.782 4.223	1171.385 160.119	1374.440 206.298
Rep + 30 min	30.500 2.665	26.860 5.060	90.048 9.006	38.133 0.572	26.167 14.757	108.000 15.192	78.000 8.579	84.202 11.031	14.385 5.641	1023.843 243.986	1268.892 149.895
Rep + 60 min	30.500 2.665	26.860 5.060	90.048 9.006	38.133 0.572	23.167 9.786	109.333 13.952	76.667 15.423	83.305 16.347	11.812 4.400	1072.743 213.981	1315.868 229.387
Rep + 90 min	30.500 2.665	26.860 5.060	90.048 9.006	38.133 0.572	24.833 11.686	117.833 17.971	84.333 15.332	91.553 15.246	11.460 2.581	1225.330 258.632	1444.763 194.683

Tot mass – total animal mass (kg)

Isch mass – ischemic segment mass (g)

Vent mass – left ventricle mass (g)

Temp – temperature (°C)

CBF – coronary bloodflow (mL/min)

HR – heart rate (min⁻¹)

MAP – mean arterial pressure (mmHg)

+dP/dt – maximal positive value of the instantaneous first derivative of left ventricular pressure (mmHg.sec⁻¹)

-dP/dt – maximal negative value of the instantaneous first derivative of left ventricular pressure (mmHg.sec⁻¹)

Pes – left ventricular pressure at the end of systole (mmHg)

Ped – left ventricular pressure at the end of diastole (mmHg)

CBF decreased during ischemia, and was increased at 10 minutes post reperfusion, compared to both control, and other post reperfusion values. By 30

minutes post reperfusion CBF had returned to control values, and would remain so until the end of the experiment

Heart rate, MAP, LV endsystolic pressure, LV end diastolic pressure, dP/dt and – dP/dt, remained unchanged during the experiment.

3.6.2 Segment data

Step	LAD L max	LAD L min ₁	LAD L min ₂	LAD L ₀	LAD Ees	LXLmax	LxL min ₁	LXL min ₂	LXL ₀	LXEes
Control	19.663 3.920	14.650 3.515	14.650 3.515	11.057 3.733	27.832 14.878	16.163 2.502	12.757 2.110	12.757 2.110	11.362 2.498	73.487 49.486
Isch	21.376 4.374	19.148 3.791	16.832 3.953	14.898 3.211	25.300 10.498	16.890 2.262	13.660 2.483	13.256 2.093	11.430 2.181	58.096 57.380
Rep + 10 min	21.440 4.227	17.685 3.979	16.760 3.559	12.050 2.670	20.673 9.975	16.673 2.068	13.330 2.107	13.330 2.107	11.623 2.315	71.338 66.638
Rep + 30 min	21.247 4.115	17.803 3.949	16.782 3.575	12.958 2.662	26.627 16.744	16.673 1.996	13.473 2.050	13.473 2.050	11.140 2.472	42.673 15.284
Rep + 60 min	20.575 3.869	17.005 3.929	16.383 3.350	12.443 2.832	22.873 7.337	16.392 2.168	13.220 1.943	13.220 1.943	11.570 3.030	46.850 18.709
Rep + 90 min	20.493 3.858	16.928 4.006	16.437 3.486	10.517 2.427	18.353 6.593	16.403 2.326	13.363 2.025	13.363 2.025	10.450 2.433	45.638 30.122

LAD Lmax – maximum LAD segment length (mm)

LAD Lmin₁ – minimum LAD segment length at the end of systole (mm)

LAD Lmin₂ – minimum LAD segment length (mm)

LAD L₀ – LAD segment length when left ventricular pressure is zero (mm)

LAD Ees – end systolic elastance of the LAD segment (mmHg.mm⁻¹)

LX Lmax – maximum circumflex segment length (mm)

LX Lmin₁ – minimum circumflex segment length at the end of systole (mm)

LX Lmin₂ – minimum circumflex length (mm)

LX L₀ – circumflex segment length when left ventricular pressure is zero (mm)

LX Ees – end systolic elastance of the circumflex segment (mmHg.mm⁻¹)

LAD segment

Maximum LAD segment length (LAD Lmax) increased during ischemia, and only decreased again at 60 minutes post reperfusion.

LAD Lo (unstressed LAD segment length) increased during ischemia, and returned to control values by 10 minutes post reperfusion.

LAD Ees remained unchanged throughout.

LX segment

LX Lmax, LXLo, and LX Ees remained unchanged during the experiments.

3.6.3 LAD segment calculations

Step	LADtot dL	LAD syst dL	LAD syst dL %	LAD PSS	LAD PSS %	LAD SW	LAD stat C
Control	5.013	5.013	25.932	0.000	0.000	373.619	2.703
	0.814	0.814	4.384	0.000	0.000	56.156	1.849
Isch	4.544	2.228	10.155	2.316	31.301	179.286	1.417
	0.646	1.075	4.604	1.087	14.619	110.088	0.350
Rep + 10 min	4.680	3.755	17.579	0.925	12.525	323.257	2.447
	1.347	1.806	7.327	0.732	9.899	177.179	2.218
Rep + 30 min	4.465	3.443	16.245	1.022	13.833	293.861	1.722
	1.334	1.674	6.922	0.623	8.434	149.792	0.789
Rep + 60 min	4.192	3.570	17.682	0.622	8.404	293.952	1.995
	1.253	1.461	6.635	0.749	10.111	129.489	0.858
Rep + 90 min	4.057	3.565	17.678	0.492	6.647	318.378	1.853
	1.395	1.790	8.130	0.738	9.960	156.580	0.516

LAD tot dL – total shortening of LAD segment (mm)
LAD syst dL – total systolic shortening of LAD segment (mm)
LAD syst dL% - LAD segment systolic shortening as a percentage of maximum LAD segment length (%)
LAD PSS – LAD segment postsystolic shortening (mm)
LAD PSS% -LAD segment postsystolic shortening as a percentage of total LAD segment shortening (%)
LAD SW – LAD segment stroke work (mm.mmHg)
LAD stat C – LAD segment static compliance (mm.mmHg⁻¹)

Total LAD segment shortening remained decreased at 90 minutes post reperfusion compared to control values.

LAD segment systolic shortening (LAD syst dL) decreased during ischemia, and remained decreased at 90 minutes post reperfusion with no improvement from ischemic values.

LAD segment systolic shortening as a percentage of maximum LAD segment length (LAD syst dL%) decreased during ischemia and even though the post ischemic values were higher than the ischemic values, control values had not been reached by the end of the experiment at 90 minutes post reperfusion.

LAD segment PSS, and PSS as a percentage of total LAD segment shortening increased during ischemia, and although the post reperfusion values were all lower than the ischemic values, control values were only reached at 60 minutes post reperfusion.

LAD segment stroke work decreased during ischemia, but had returned to control values by 10 minutes post reperfusion.

LAD segment static compliance remained unchanged during the experiments.

3.6.4 Circumflex segment calculations

Step	LX tot dL	LX syst dL	LX syst dL%	LX PSS	LX PSS%	LX SW	LX stat C
Control	3.407	3.407	21.076	0.000	0.000	253.270	2.133
	0.785	0.785	4.150	0.000	0.000	52.989	1.216
Isch	3.634	3.230	19.335	0.404	10.636	244.703	1.144
	1.364	1.200	7.023	0.653	14.615	113.247	0.338
Rep + 10 min	3.343	3.343	20.165	0.000	0.000	275.562	1.803
	0.947	0.947	5.387	0.000	0.000	85.723	1.367
Rep + 30 min	3.200	3.200	19.311	0.000	0.000	270.283	1.293
	0.874	0.874	5.016	0.000	0.000	82.196	0.433
Rep + 60 min	3.172	3.172	19.371	0.000	0.000	264.943	1.507
	0.720	0.720	3.774	0.000	0.000	82.095	0.449
Rep + 90 min	3.040	3.040	18.485	0.000	0.000	277.142	1.500
	0.854	0.854	4.345	0.000	0.000	85.420	0.430

LX tot dL – LX segment total shortening (mm)

LX syst dL – LX segment total systolic shortening (mm)

LX syst dL% - LX segment systolic shortening as a percentage of maximum LX segment length (%)

LX PSS – LX segment post systolic shortening (mm)

LX PSS% - LX segment post systolic shortening as a percentage of total LX segment shortening (%)

LX SW – LX segment stroke work (mm.mmHg)

LX stat C – LX segment static compliance (mm.mmHg⁻¹)

LX tot dL; LX syst dL, LX syst dL%, LX PSS, LX PSS% and LX stat C remained constant throughout the experiment.

3.6.5 Bloodgas data

Step	pH a	PaCO ₂	PaO ₂	HCO ₃ a	SaO ₂	Hb	pH v	PvCO ₂	PvO ₂	HCO ₃ v	SvO ₂	lac a	lac v
Control	7.400 0.031	5.852 1.281	29.683 11.201	30.733 5.244	98.833 1.659	10.250 1.084	7.342 0.040	7.377 1.285	3.517 0.462	33.417 3.679	46.650 9.866	0.867 0.393	0.883 0.652
Isch	7.384 0.054	5.248 0.826	33.840 6.497	27.540 2.345	99.660 0.391	10.300 1.204	7.214 0.050	8.060 0.911	4.660 2.196	27.380 4.470	51.300 19.264	0.740 0.602	3.140 1.566
Rep + 10 min	7.400 0.072	5.702 1.231	32.258 9.166	29.100 5.172	99.533 0.476	10.250 1.084	7.285 0.067	6.540 1.380	5.797 1.646	29.733 3.886	73.933 17.908	1.083 0.527	1.700 0.369
Rep + 30 min	7.385 0.043	5.663 0.675	33.680 7.166	28.900 5.017	99.633 0.472	10.250 1.084	7.330 0.076	6.723 1.496	4.688 1.323	30.867 3.702	62.517 19.557	1.050 0.497	1.417 0.776
Rep + 60 min	7.390 0.046	5.610 0.799	31.197 7.866	29.350 4.948	99.650 0.351	10.250 1.084	7.272 0.135	7.035 1.108	4.310 0.846	31.350 2.995	58.117 14.833	1.033 0.520	1.202 0.701
Rep + 90 min	7.392 0.048	5.517 0.918	33.437 7.609	28.683 5.061	99.667 0.372	10.250 1.084	7.320 0.070	7.003 1.075	4.128 1.015	30.883 2.849	54.833 17.629	1.083 0.214	1.483 0.621

pH a – arterial pH

PaCO₂ – arterial PCO₂ (kPa)Pa O₂ – arterial PO₂ (kPa)HCO₃a – arterial HCO₃ (mmol.ℓ⁻¹)SaO₂ – arterial saturation (%)Hb – hemoglobin (g.dℓ⁻¹)

pH v – coronary venous pH

PvCO₂ – coronary venous PCO₂ (kPa)PvO₂ – coronary venous PO₂ (kPa)HCO₃ v – coronary venous HCO₃ (mmol.ℓ⁻¹)SvO₂ – coronary venous saturation (%)lac a – arterial lactate (mmol.ℓ⁻¹)lac v – venous lactate (mmol.ℓ⁻¹)

Hb remained unchanged during the experiment. Coronary venous lactate increased during ischemia, but returned to control values by 10 minutes post reperfusion.

3.6.6 Bloodgas calculations

Step	CaO ₂	CvO ₂	a-v dO ₂	a-v dO ₂ %	$\dot{V}O_2$	$\dot{V}O_2/100g$	$\dot{V}O_2/100g/$ beat	CBF/100g	CBF/100g/ beat	a-v d lact
Control	14.776	6.787	7.989	54.584	1.959	7.740	0.072	94.688	0.885	-0.017
	1.748	1.812	0.973	8.606	0.474	3.203	0.031	29.045	0.300	0.821
Isch	15.052	7.339	7.713	50.341	0.152	0.488	0.005	4.763	0.046	-2.400
	1.753	2.313	3.154	19.250	0.324	1.031	0.010	9.812	0.095	1.663
Rep + 10 min	14.929	10.794	4.135	28.683	1.461	5.679	0.052	158.147	1.418	-0.617
	1.611	3.179	1.993	16.717	0.234	1.775	0.015	68.962	0.457	0.445
Rep + 30 min	14.974	9.064	5.910	39.929	1.322	5.154	0.047	96.901	0.884	-0.367
	1.555	2.996	2.523	18.312	0.426	2.206	0.016	49.956	0.370	0.709
Rep + 60 min	14.920	8.438	6.482	43.905	1.412	5.453	0.049	86.211	0.781	-0.168
	1.578	2.517	1.889	14.024	0.439	2.178	0.015	32.203	0.241	0.549
Rep + 90 min	14.975	7.970	7.005	47.294	1.588	6.155	0.053	92.512	0.770	-0.400
	1.560	2.814	2.284	16.587	0.540	2.608	0.023	39.380	0.253	0.569

CaO₂ – arterial oxygen content (mL.100mL⁻¹)

CvO₂ – coronary venous oxygen content (mL.100mL⁻¹)

a-v dO₂ – arterial-venous oxygen content difference (mL.100mL⁻¹)

a-v dO₂% - arterial-venous oxygen content difference as a percentage of arterial oxygen content (%)

$\dot{V}O_2$ – oxygen consumption in LAD segment (mL.min⁻¹)

$\dot{V}O_2/100g$ – oxygen consumption per 100g LAD segment tissue (mL.min⁻¹)

$\dot{V}O_2/100g/beat$ – oxygen consumption per 100g LAD segment tissue per beat (mL)

CBF/100g – coronary blood flow per 100g LAD segment tissue (mL.min⁻¹)

CBF/100g/beat – coronary blood flow per 100g LAD segment tissue per beat (mL)

a-v d lact – difference in arterial and coronary venous lactate (mmol.L⁻¹)

Arterial oxygen content remained unchanged throughout the experiment.

VO₂, VO₂/100g and VO₂/100g/beat in the LAD segment decreased during ischemia, and although it increased from ischemic values from 10 minutes post

reperfusion onwards, it only reaches control values by 90 minutes post reperfusion.

CBF/100g and CBF/100g/beat in the LAD segment decreased during ischemia, and increased at 10 minutes post reperfusion to values higher than both control, and other post reperfusion values. By 30 minutes post reperfusion CBF/100 and CBF/100g/beat was the same as control values and remained so.

The difference in arterial and coronary venous lactate increased during ischemia and returned to control values by 10 minutes post reperfusion.

3.7 Reperfusion arrhythmias

	n	VF	VES	Joules	Lignocaine
Verapamil 2mg	13	0	5	0	190 mg
Saline	15	6	8	265	810 mg
Verapamil 0.5 mg	8	4	3	100	345 mg

	n	VF%	VES%	Joules/animal	Lignocaine/animal
Verapamil 2mg	13	0	38.46	0	14.62 mg
Saline	15	40	53.33	17.67	54 mg
Verapamil 0.5 mg	8	50	37.5	12.5	43.13 mg

n - number of animals in the group.

VF - number of animals developing ventricular fibrillation on reperfusion.

VES – number of animals developing ventricular extrasystoles on reperfusion.

Joules – total joules used for defibrillation according to protocol.

Lignocain – total mg amount of lignocain used according to protocol in

that group.

Joules/animal – total joules for that group divided by the number of animals in the group.

Lignocain/animal – total mg amount of lignocain used divided by the number of animals in the group.

VF% - percentage of animals in the group developing VF (%).

VES% - percentage of animals in the group developing VES (%).

Animals receiving 2mg of verapamil, either at the onset of ischemia or 3 minutes prior to reperfusion had a significantly lower incidence of ventricular fibrillation than animals in the saline groups.

There was no difference in the incidence of ventricular fibrillation or ventricular extrasystoles between the saline groups and the group receiving 0.5mg of verapamil.

3.8 Evidence of ischemia

Data in this section was obtained from all animals subjected to ischemia (n = 36).

3.8.1 Myocardial function

	LAD L max	LAD Lo	LAD syst dL	LAD syst dL%
Control value	19.05 0.06	10.6 1.6	4.89 0.78	25.64 3.64
Ischemic value	21.6 0.27	16.57 0.89	1.62 1.17	7.68 4.72
t- value	-6.6	11.08	11.08	12.5
p-value	<0.0000	<0.0000	<0.0000	<0.0000

	LAD PSS	LAD PSS%	LAD SW	LAD Ees
Control value	0.08 0	2.06 0	38422 103.21	29.57 1.09
Ischemic value	2.11 0.19	52.65 40.07	124.74 77.03	45.74 4.26
t- value	-8.7	-9.01	9.08	-1.96
p-value	<0.0000	<0.0000	<0.0000	<0.06

LAD L max – maximum LAD segment length (mm)
LAD L_o – LAD segment length when left ventricular pressure is zero (mm).
LAD syst dL – total systolic shortening of LAD segment (mm).
LAD syst dL% - LAD segment systolic shortening as a percentage of

maximum LAD segment length.

LAD PSS – LAD segment postsystolic shortening (mm)

LAD PSS% - LAD segment postsystolic shortening as a percentage of total LAD segment shortening (%).

LAD SW – LAD segment stroke work (mm.mmHg).

LAD Ees – end systolic elastance of the LAD segment (mmHg.mm⁻¹)

3.8.2 Myocardial metabolism

	pH _v	PvCO ₂	PvO ₂	HCO ₃
Control value	7.34 0.08	7.78 2.67	3.46 0.0	33.48 1.2
Ischemic value	7.25 0.02	9.72 1.77	2.81 0.8	30.51 0.14
t- value	6.88	-4.20	-15.642	4.42
p-value	<0.0000	<0.0000	<0.0000	<0.0001

	SvO ₂	lac a	lac v	dlact%
Control value	40.02 9.9	0.63 0.57	0.57 0.71	-51.10 8.84
Ischemic value	36.51 8.06	0.66 0.92	2.97 0.21	-1465 35.88
t- value	No difference	No difference	-6.10	4.13
p-value			<0.0000	<0.0001

	CBF	CBF/100g	$\dot{V}O_2$	$\dot{V}O_2/100g$
Control value	27.13 7.78	73.76 26.54	2.47 0.85	6.61 2.74
Ischemic value	1.58 5.59	4.08 14.27	0.17 0.46	0.42 1.18
t- value	15.66	13.62	13.94	13.04
p-value	<0.0000	<0.0000	<0.0000	<0.0000

pH_v – coronary venous pH.

PvCO₂ – coronary venous PCO₂ (kPa).

PvO₂ – coronary venous PO₂ (kPa).

HCO_{3v} – coronary venous HCO₃ (mmol.l⁻¹).

SVO₂ – coronary venous saturation (%).

lac a – arterial lactate (mmol.l⁻¹).

lac v – coronary venous lactate (mmol.l⁻¹).

a-v d lact% – difference in arterial and coronary venous lactate as a percentage of arterial lactate (%).

CBF – coronary blood flow (ml.min⁻¹).

CBF/100 g – coronary blood flow per 100g LAD segment tissue ml.min⁻¹).

$\dot{V}O_2$ – oxygen consumption in the LAD segment (ml.min⁻¹).

$\dot{V}O_2/100g$ – oxygen consumption per 100g LAD segment tissue (ml.min⁻¹).

There was a significant increase in maximum LAD segment length and unstressed LAD segment length during ischemia.

LAD segment systolic shortening and LAD segment systolic shortening as a percentage of maximum LAD segment length decreased significantly during ischemia.

There was a significant increase in LAD segment postsystolic shortening and postsystolic shortening as a percentage of total LAD segment shortening.

LAD segment stroke work decreased during ischemia.

Coronary venous pH, PO_2 and HCO_3 all decreased significantly during ischemia.

There was a significant increase in coronary venous PCO_2 , lactate, and the difference in arterial and venous lactate as a percentage of arterial lactate.

CBF, CBF/100g, $\dot{V}O_2$ and $\dot{V}O_2/100g$ all decreased significantly during ischemia.

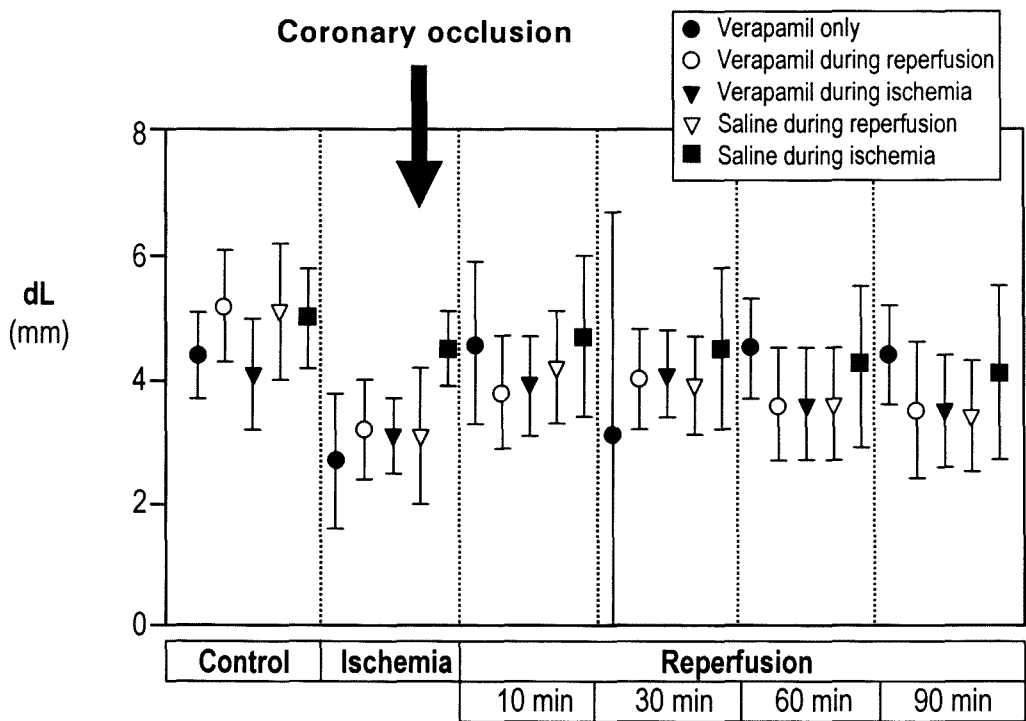


Fig. 3.1

Changes in **regional systolic shortening** during experiments.
Values are the mean / SDM.
Note verapamil *only* group did not undergo coronary occlusion.

In all the LAD **occlusion groups** dL was significantly less during ischemia compared to control.
The apparent decrease in dL in the **verapamil only** group did not reach statistical significance.
Verapamil during reperfusion: dL returned to control by 30 minutes reperfusion.
Verapamil during ischemia: dL returned to control by 10 minutes reperfusion.
In both the **saline groups:** dL did not return to control values for the duration of the reperfusion.

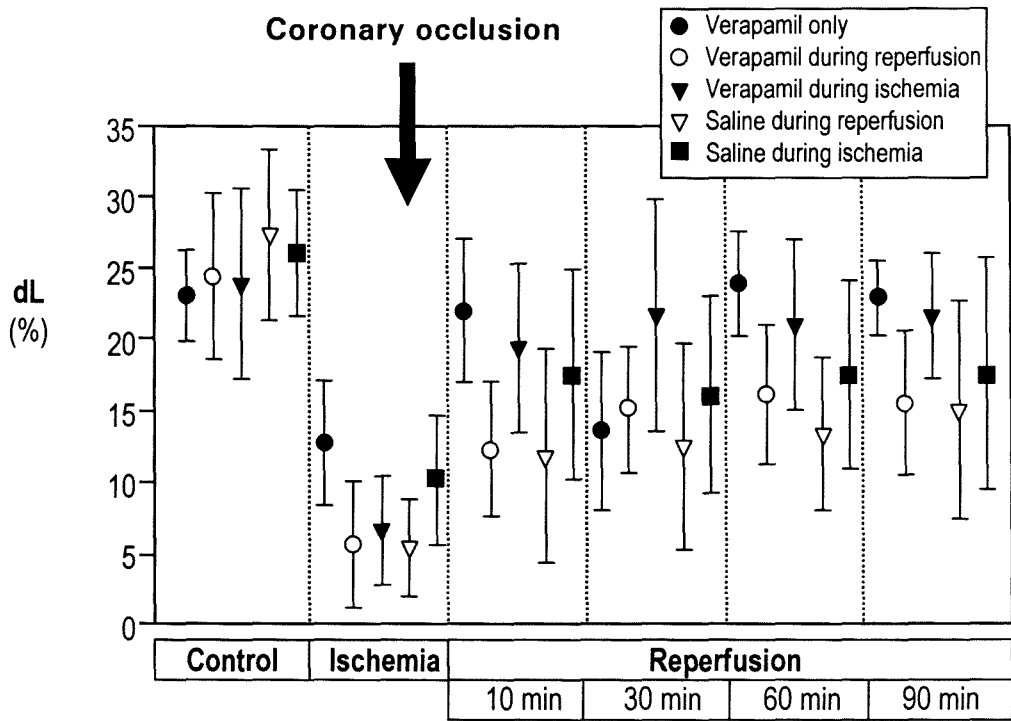


Fig. 3.2

Changes in **regional normalized systolic shortening** during experiments. Values are the mean / SDM.

Note verapamil *only* group did not undergo coronary occlusion.

In all the LAD **occlusion groups** dL% was significantly less during ischemia compared to control.

The apparent decrease in dL% in the **verapamil only** group did not reach statistical significance.

Verapamil during reperfusion: dL% returned to control by 30 minutes reperfusion.

Verapamil during ischemia: dL% returned to control by 10 minutes reperfusion.

In both the **saline groups**: dL% did not return to control values for the duration of the reperfusion.

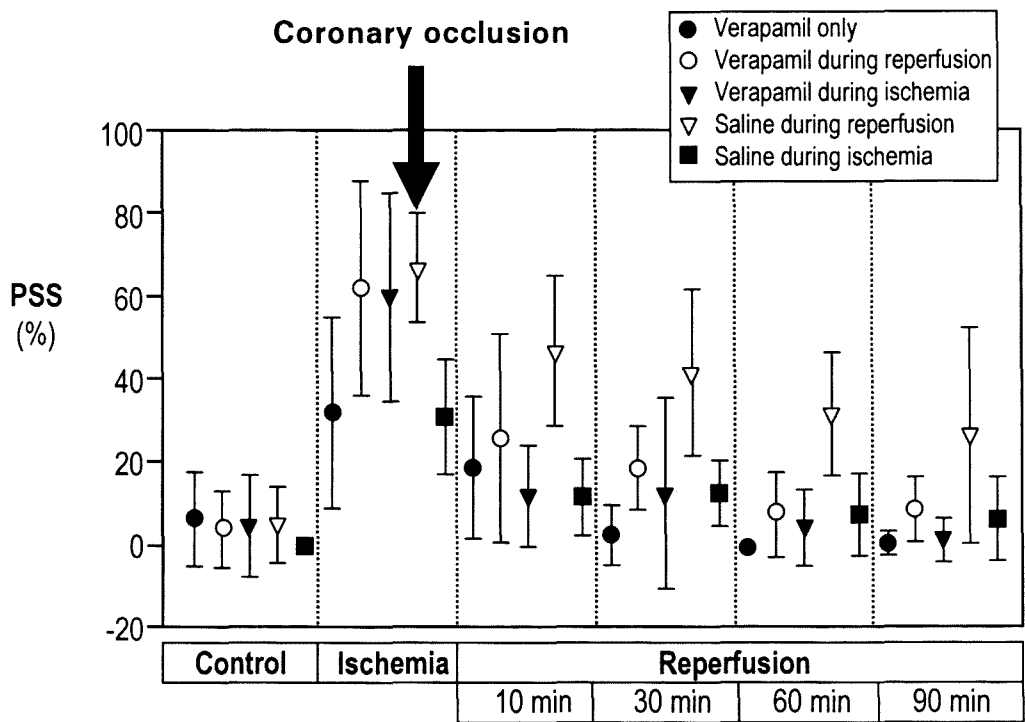


Fig. 3.3

Changes in **percent post systolic shortening** during experiments. Values are the mean / SDM.
Note verapamil only group did not undergo coronary occlusion.

PSS increased form control in **all the groups** where occlusion was applied to the LAD.
Verapamil only: PSS increased after infusion and returned to control by 30 minutes after reperfusion.
In verapamil during ischemia and reperfusion: PSS returned to controls by 10 minutes reperfusion.
In saline during reperfusion: PSS did not return to control for the rest of the experiment.
Saline during ischemia: PSS was back to control by 60 minutes reperfusion.

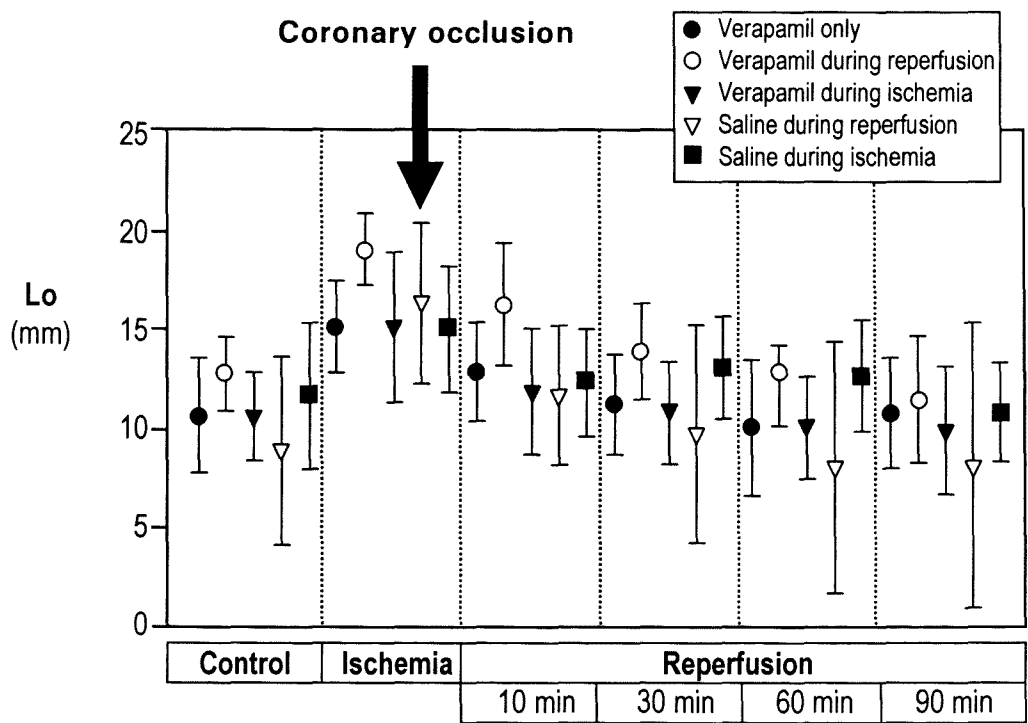


Fig. 3.4

Changes in **extrapolated regional segment length at the time LV pressure is zero** during experiments.

Values are the mean / SDM.

Note verapamil *only* group did not undergo coronary occlusion.

Verapamil only : Lo was increased after infusion and returned to control by 10 minutes reperfusion.

Verapamil during reperfusion: Lo was raised during occlusion and returned to control by 30 minutes reperfusion.

Verapamil during ischemia: Lo increased during ischemia and returned to control by 10 minutes reperfusion.

Saline groups: Lo increased during ischemia and returned to control by 10 minutes reperfusion.

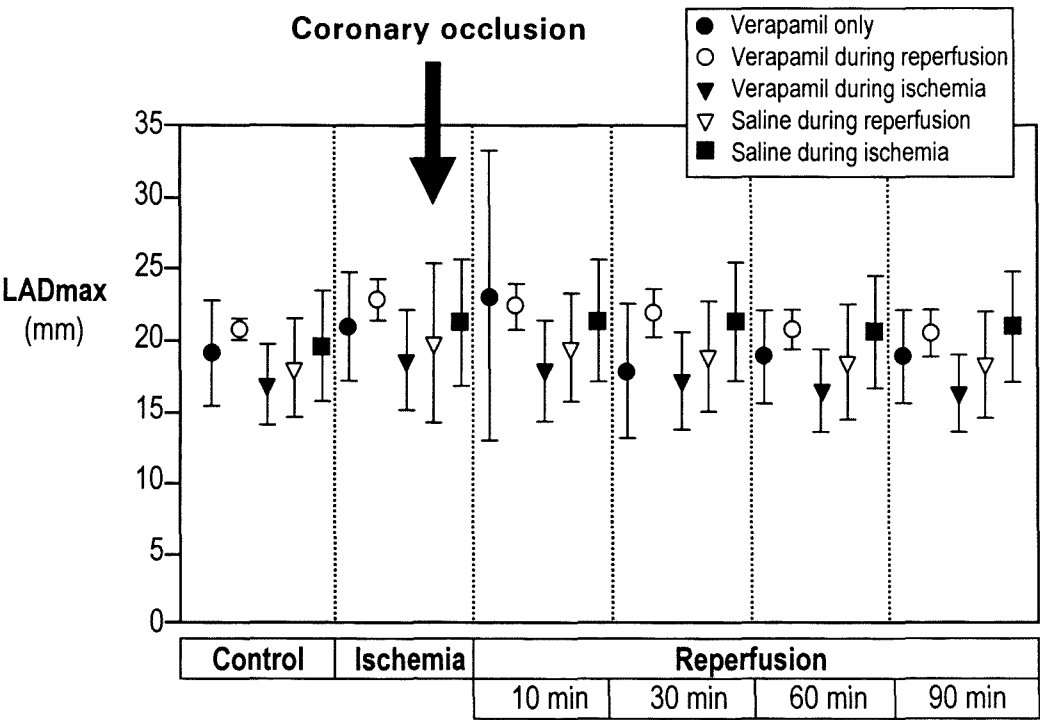


Fig. 3.5

Changes in **regional segment length at end-diastole** during experiments.

Values are the mean / SDM.

Note verapamil *only* group did not undergo coronary occlusion.

In **all** the groups LADmax increased during ischemia.

Verapamil only: LADmax retruned to control by 30 minutes.

Verapamil during reperfusion : LADmax returned to control by 30 minutes reperfusion.

Verapamil during ischemia: LADmax retruned to control by 10 minutes.

Saline groups: LADmax returned to control by 60 minutes reperfusion.

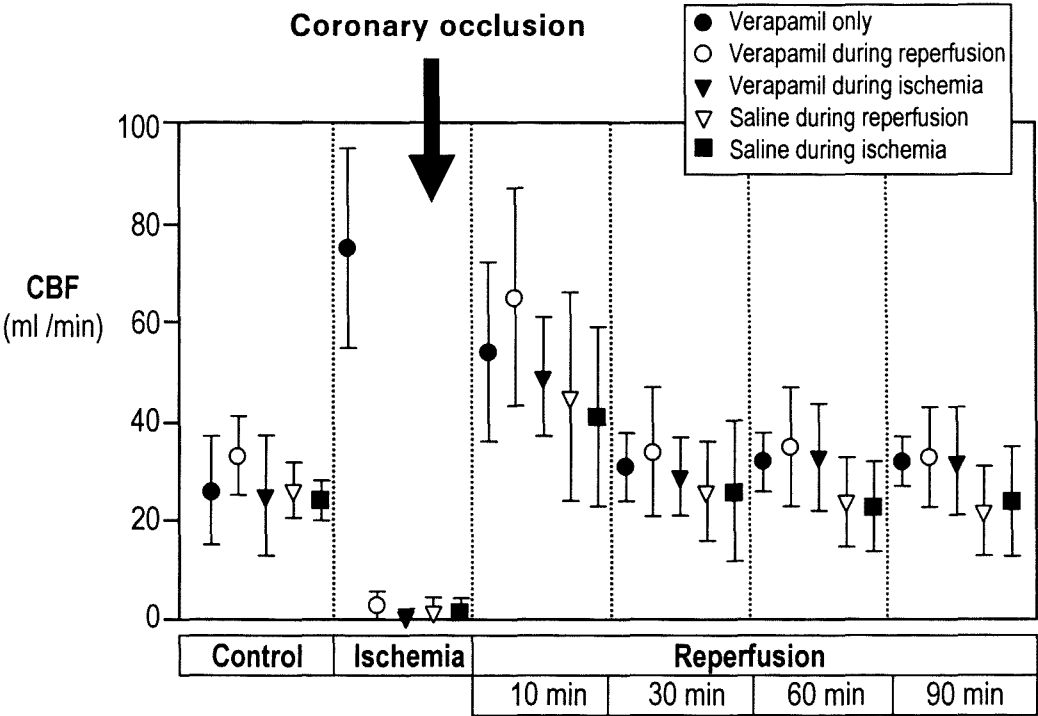


Fig. 3.6

Changes in **coronary blood flow** during experiments. Values are the mean / SDM.

Note verapamil *only* group did not undergo coronary occlusion.

CBF decreased in all the groups on occlusion of the LAD artery except for the verapamil only group where no occlusion was applied.

Verapamil only: CBF increased during infusion and remained higher than control for the rest of the study.

All the other groups: CBF was raised at the 10 minute reperfusion measurement. At 30 minutes reperfusion it was no different from control.

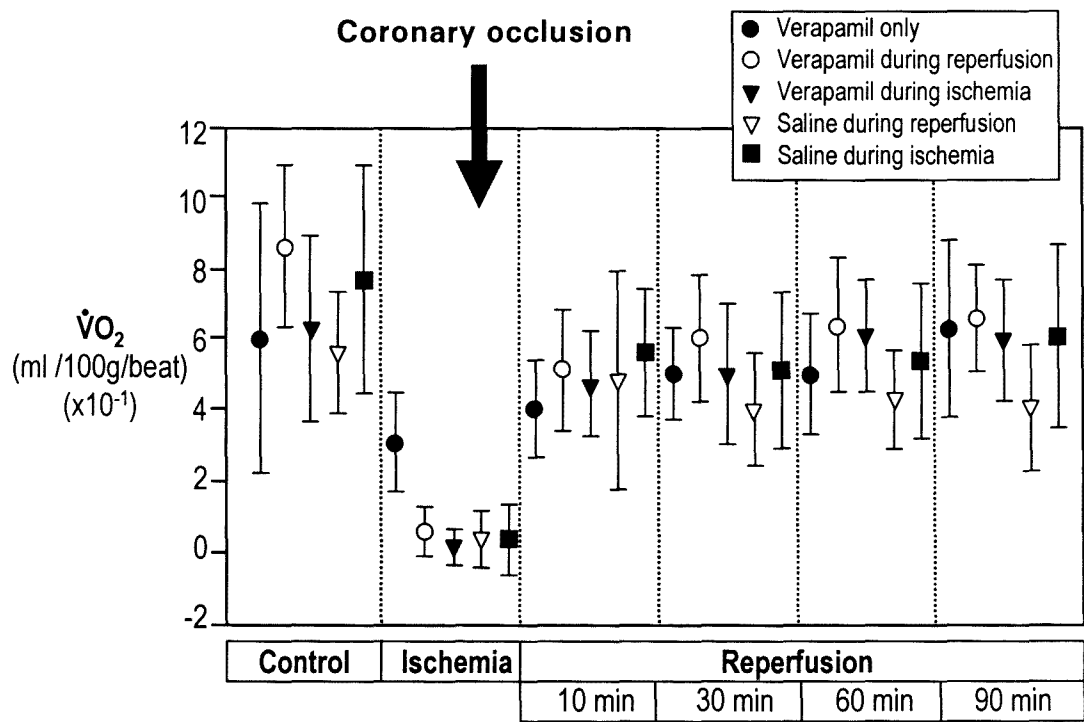


Fig. 3.7

Changes in **regional oxygen consumption** during experiments.
Values are the mean / SDM.
Note verapamil *only* group did not undergo coronary occlusion.

In all the **occlusion experiments**, the oxygen consumption was significantly reduced on occlusion of the coronary artery.

Verapamil only: Oxygen consumption decreased after infusion and returned to control values by 10 minutes after infusion.

Verapamil only: Oxygen consumption was no different from control at 10 minutes.

Verapamil during ischemia and reperfusion groups: The oxygen consumption was no different from controls at 10 minutes reperfusion.

Saline during reperfusion group: Oxygen consumption was no different from control by 90 minutes reperfusion.

Saline during ischemia group: Oxygen consumption was no different from control by 10 minutes reperfusion.

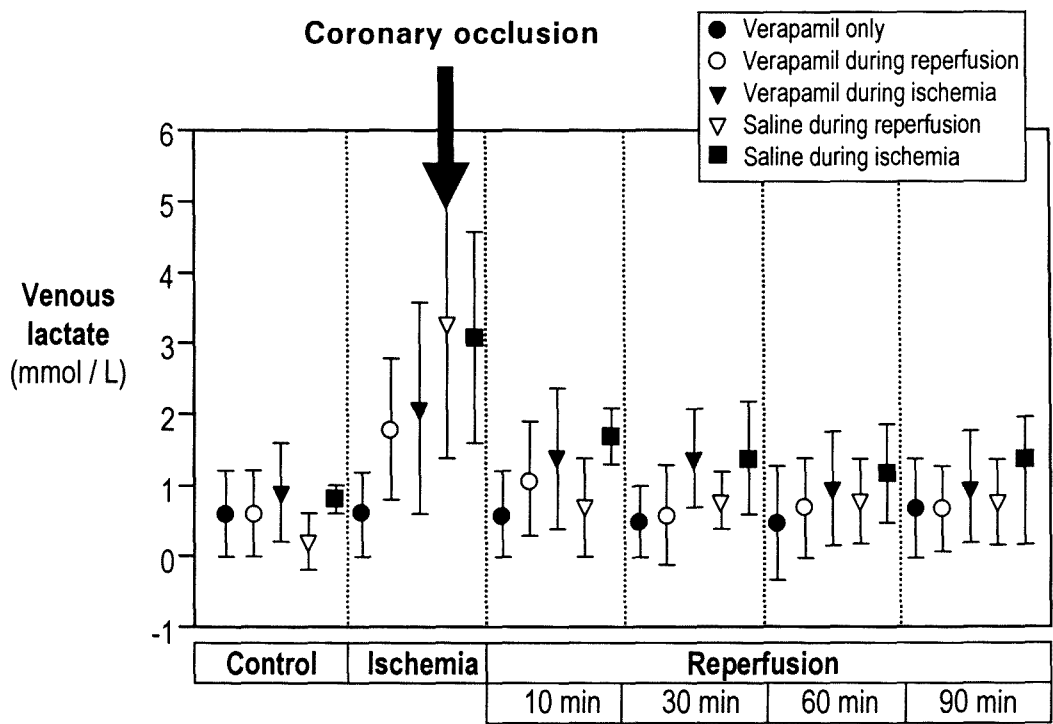


Fig. 3.8

Changes in **venous lactate** during experiments. Values are the mean / SDM.

Note verapamil *only* group did not undergo coronary occlusion.

In the **verapamil only** the lactate did not increase during ischemia.
All the other groups showed an increase during ischemia.
In al the **other groups** the lactate concentration was back to control by 10 minutes reperfusion.

4. Discussion

4.1 Introduction.

4.2 Discussion of the experimental model.

4.2.1 The use of the porcine as opposed to the canine model.

4.2.2 The difference between anesthetized open chest preparations and conscious preparations.

4.2.3 The duration of ischemia.

4.2.4 Intracoronary administration of verapamil.

4.2.5 Timing of drug administration.

4.2.6 The dosage of verapamil.

4.2.7 The anesthetic technique.

4.3 Evidence of myocardial ischemia.

4.3.1 Functional evidence of myocardial ischemia.

4.3.2 Metabolic evidence of myocardial ischemia.

4.4 Evidence of reperfusion.

4.5 The characteristic reperfusion injury.

4.6 Verapamil and the reperfusion injury.

4.7 The effect of verapamil on reperfusion arrhythmias.

4.1 Introduction.

The reperfusion injury and specifically stunning, poses a problem in the clinical setting after coronary artery revascularization. Although calcium channel blocking drugs have been shown to attenuate the reperfusion injury (Opie 1992, Przyklenk 1992, Nayler 1991), the systemic administration of these drugs in the above clinical setting is often problematic. This is due to the adverse hemodynamic effects resulting from a dose large enough to be effective in attenuating the reperfusion injury.

The aim of this thesis is to establish whether verapamil, administered timeously, directly into the coronary artery supplying the ischemic area, at a dose large enough to be effective locally, but without systemic effects, will attenuate calcium induced hypercontracture and the resultant cardiac dysfunction during reperfusion.

4.2 Discussion of the experimental model.

Factors of importance in this experimental model include:

- The animal species used, and the degree of collateral coronary artery bloodflow.
- Whether the animal was conscious or the anesthetized open chest model.
- The duration of ischemia.

- The timing, dosage and location of drug administration.

4.2.1 The use of the porcine as opposed to the canine model.

No animal model will meet all the relevant criteria or be an ideal choice, however, the one selected should most closely resemble the experimental objective and natural history of the human counterpart (Dodds 1982). The pig has become a widely used and important large animal model for research on human disease. An advantage of this model is that the cardiovascular system is similar in anatomy and hemodynamics to that of humans (Bustad 1968).

As opposed to the canine model, the porcine heart resembles the human heart in respect of the lack of native coronary collateral vessels and xanthine oxidase activity (Eckstein 1954, White and Bloor 1981, Bloor 1986, White 1986, Muxfeldt and Schaper 1987, Podzuweit 1987, Roth 1987 and Grum 1989). The paucity of collateral vessels in the porcine heart eliminates the variability in collateral flow that is inherent in canine models. Collateral flow is the major determinant of the severity of myocardial stunning (Bolli 1988), and the elimination of this variable results in a more reproducible post ischemic dysfunction among different animals.

The canine left ventricle is relatively large and has a left dominant coronary artery supply with four times more collaterals present. These collaterals are also larger than any in the pig heart. The capillary density of the left ventricle of the

dog is significantly greater than that of swine breeds (White 1986). Coronary flow at maximal heart rate is significantly higher in the dog than in the pig. This reflects either the higher heart weight to body weight ratio of the dog, or the greater vascular capacity of the dog heart. During coronary artery occlusion, collateral blood flow to the dog heart is five times greater than that of the pig (White 1986). The result of this is that after acute left circumflex coronary artery occlusion in pigs, the infarct size expressed as a percentage of the left circumflex perfusion bed is $73 \pm 4\%$. After acute LAD occlusion the infarct size as a percentage of the LAD perfusion bed is $94 \pm 3\%$. In dogs subjected to similar acute coronary artery occlusions the infarct size was only $53 \pm 7\%$ of the left circumflex perfusion bed and $61 \pm 6\%$ of the LAD perfusion bed (White 1986).

The porcine model was selected for this study for the following reasons:

- The coronary collateral flow in the pig heart is sparse, similar to that of man and less than one fourth that of the dog (White 1986). Acute occlusion of the coronary artery will eventually result in infarction of most of the vascular bed at risk.
- The coronary artery anatomy is similar to the human heart, as is the maximum coronary blood flow.
- The heart size and its relative weight to body weight is similar to man.
- Pigs are readily available at relatively low cost.

4.2.2 The differences between anesthetized open chest preparations and conscious preparations

Several investigators (Vatner 1971, Cobb 1974, Vatner and Braunwald 1975, Templeton 1975, Manders and Vatner 1976, Jugdutt 1985, Bolli 1986, Laxon 1989, Ning 1990) have pointed out that observations in open chest models may be confounded by the effects of anesthesia, surgical trauma, abnormal hemodynamic conditions, and excessive levels of circulating catecholamines. It has also been shown that in the 15 minute coronary occlusion model of myocardial stunning, both the severity of the post ischemic dysfunction (Triana 1991) and the magnitude of the associated free radical generation (Li 1993) are greatly exaggerated in open chest preparations as opposed to conscious animals.

Li et al (1993) showed that a brief episode of regional myocardial ischemia in the conscious unsedated dog is associated with the generation of free radical species on reperfusion. However this free radical production is five times less than that in the open chest anesthetized model. The magnitude of radical generation is directly related to the magnitude of subsequent depression of contractility. It follows then that after reperfusion the recovery of contractile function in the conscious animals was considerably greater than in the anesthetized open chest preparations. In Li's study the post ischemic functional depression associated with the anesthetized model was more than twice that observed in the awake model. Alternatively, approximately half of the post ischemic depression of contractility observed in the anesthetized open chest model was absent in the conscious animal.

The production of free radicals in open chest animals has two components: A smaller component caused by ischemia and reperfusion, and a larger component caused by factors unrelated to ischemia, such as trauma and adrenergic activity (Li 1993). In open chest animals, recovery of function ceases at one hour of reperfusion, no further improvement takes place between one and four hours of reflow. In contrast, in the conscious animal, recovery continues unabated throughout the first six hours of reperfusion until complete normalization is achieved (Triana 1991) (Fig. 4.1).

Triana (1991) showed that the stunned myocardium is sensitive to variations in temperature. There is an inverse relationship between the temperature and post ischemic recovery in the open chest anesthetized model. Temperature increases in the 34° tot 37°C range exert a selective depressant effect on the mechanical function of the stunned myocardium (Fig. 4.2). This finding emphasizes the importance of carefully controlling temperature in the open chest preparations of myocardial stunning. In the present study the temperature of the individual animals varied between 36 and 39°C and this may well have affected the severity of stunning, however there was no difference in the average temperature between the various groups, hence it is presumed that all groups would have been equally affected.

In this study the anesthetized open chest model was employed in all the groups, thus any exaggeration of myocardial stunning, abnormal hemodynamic

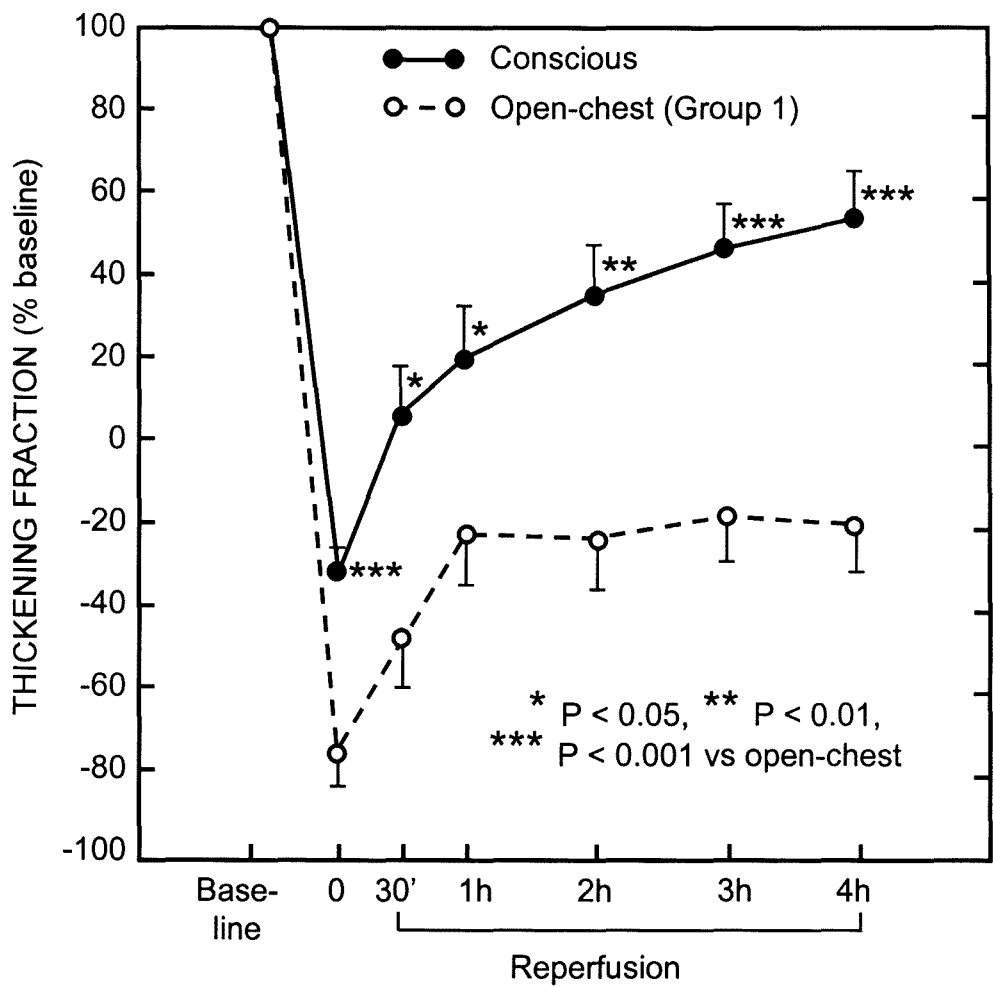


Figure 4.1

Systolic thickening fraction in a myocardial region during ischemia and reperfusion. Thickening fraction expressed as percent of baseline values. Data are mean/SDM.

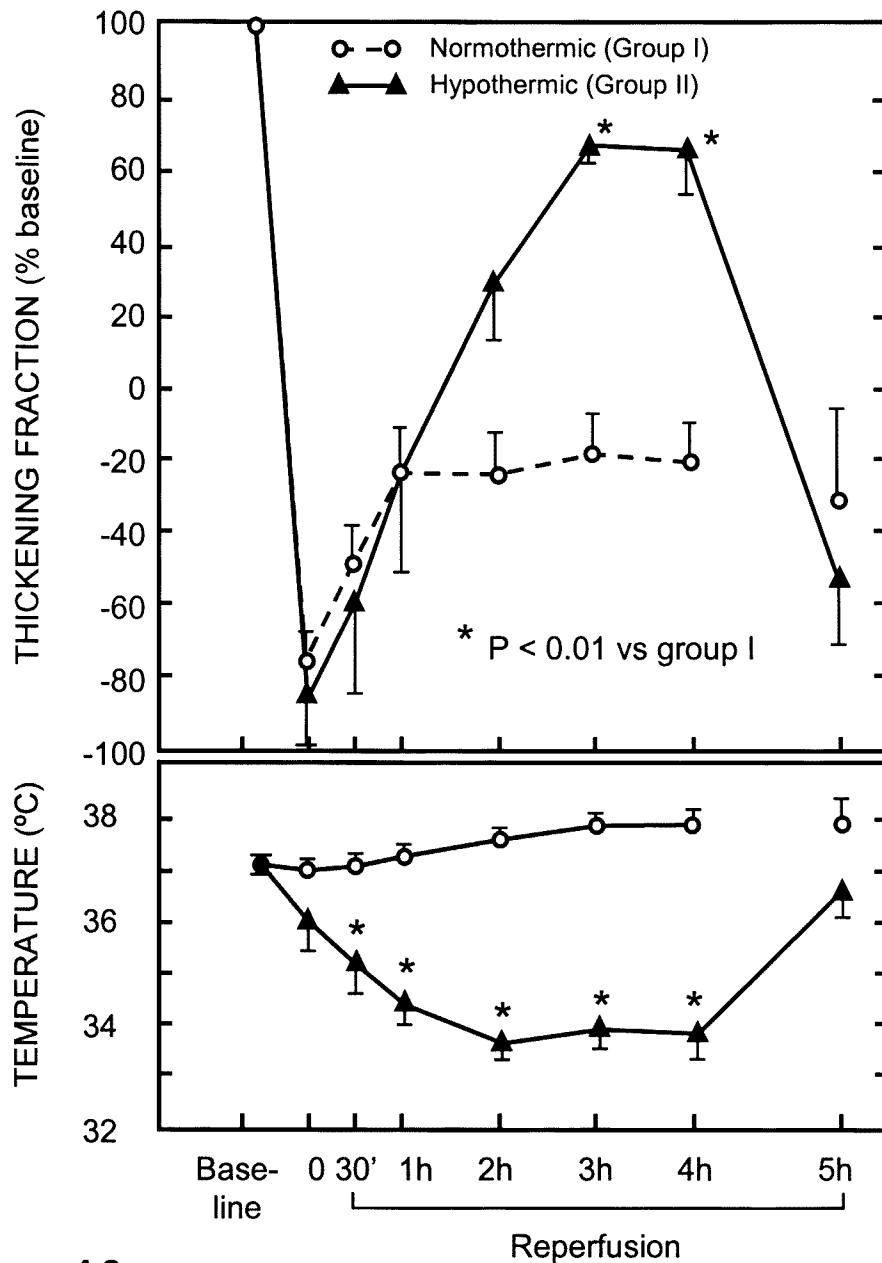


Figure 4.2

Systolic thickening fraction in ischemic / reperfused myocardium in normo and hypothermic dogs. Thickening fraction is expressed as percentage of baseline. Values are mean / SDM. Upper graph illustrates the systolic function and lower graph depicts the esophageal temperature. Temperatures were manipulated with a heating blanket.

JF Triana Circ Res 1991; 69: 731 - 747

conditions or excessive levels of catecholamines, would have affected all groups equally.

Myocardial stunning is often a problem in the clinical setting after coronary artery bypass grafting. These patients, like the animals in this study, are subjected to myocardial ischemia in the operative setting, under anesthesia, with the chest open. Hence, although the severity of myocardial stunning is greatly exaggerated in the surgical setting of the anesthetized open chest preparation (Triana 1991), this model is applicable to the clinical setting, specifically in the patient after cardiopulmonary bypass.

4.2.3 Duration of ischemia.

Previous studies have shown that calcium antagonists enhance recovery of ventricular function (Lamping and Gross 1985, Przyklenk and Kloner 1988, Warltier 1988, Taylor 1990, Ehring 1992, Gross and Pieper 1992). It is however often difficult to discern how much of the dysfunction seen in these models is due to stunning, and how much is due to infarction. This is because long periods of ischemia were used in reported studies.

Myocardial cells rendered anoxic by sudden complete occlusion of the coronary artery, cease to contract within 60 seconds (Sayen 1958). Such cells stay viable for some time, as shown in dogs by the fact that restoration of blood flow at any time during the first 18 minutes of ischemia is followed quickly by

resumption of normal cellular structure, function and metabolism (Jennings 1963). Thus, affected cells are injured by ischemia, but the injury is “reversible” provided blood flow is restored within this period. Further prolongation of ischemia beyond 18 minutes results in death of an increasing number of affected cells, irrespective of whether or not their blood supply is restored. Injury to these cells is “permanent” (Jennings 1963).

Mortally injured myocardial cells are first apparent after twenty minutes of ischemia. 55% of ischemic cells are dead after 45 minutes, and almost 100% after 60 minutes of ischemia (Jennings 1965).

Jennings (1965) showed reversible changes induced by coronary occlusion lasting 15 minutes or less (Fig. 4.4). These included: moderate relaxation of myofibers, margination of nuclear chromatin, moderate glycogen depletion, and loss of cristae and less dense matrices in occasional mitochondria. However, as in non-ischemic (control) myocardium, intra mitochondrial granules were not seen, and the sarcolemma, intercalated disks, sarcoplasmic reticulum and transverse tubular system were normal, as was capillary morphology.

If however ischemia lasts 30 minutes or longer, myocardial cells were irreversibly injured. This is demonstrated by extreme relaxation of myofibers, more marked margination of nuclear chromatin, a virtual absence of glycogen, and extensive mitochondrial abnormalities. Intra mitochondrial granules, representing calcium accumulations, became increasingly prominent with more prolonged ischemia.

Disruptions and discontinuities of the sarcolemma and sarcoplasmic reticulum occurred after 60 minutes of ischemia (Fig. 4.3, 4.5, 4.6).

Heyndrickx (1975) demonstrated that when reperfusion occurred after 15 minutes of coronary artery occlusion, electrographic changes revert to normal within 1 minute and necrosis does not ensue. However, regional myocardial function in the previously ischemic zone of the heart remains depressed for at least 6 hours. Regional function does recover completely between 6 and 24 hours post reperfusion, which is consistent with the concept that no permanent damage has occurred. Hence, the threshold for irreversible myocardial injury is reached at approximately 20 minutes of ischemia, at which point occasional small foci of necrosis result (Jennings 1965, Reimer 1977). A coronary occlusion period of 15 minute duration results in structural and functional alterations that are ultimately fully reversible (Heyndrickx 1977, 1978).

In the present study an ischemic period of 15 minutes was chosen as this ensures the presence of the reperfusion injury (stunning and dysrhythmias) without resulting in irreversible myocardial damage.

4.2.4 Intracoronary administration of verapamil

Systemic administration of any one of several calcium channel blocking drugs (verapamil, diltiazem, nifedipine, nitrendipine, amlodipine) has been shown to improve recovery of function in regionally stunned myocardium in intact animals



Figure 4.3

Myocardial cell after 60 minute coronary artery occlusion. The myofibrils are moderately contracted, the nuclear and sarcolemmal membranes are scalloped. Transverse tubules (t), which are morphologically similar to the sarcolemma, are seen at or near to the Z bands. The granular chromatin of the nucleus (n) is evenly dispersed and the nucleolus (nu) prominent. The mitochondriae (m) have moderately dense matrices, normal cristae and do not contain dense granules. The glycogen (g) content is normal.



Figure 4.4

Fifteen minutes of ischemia. The nucleus (n) shows dispersion and some margination of chromatin material. The amount of glycogen is diminished.

RB Jennings Arch Pathol 1965: 79: 135 - 143

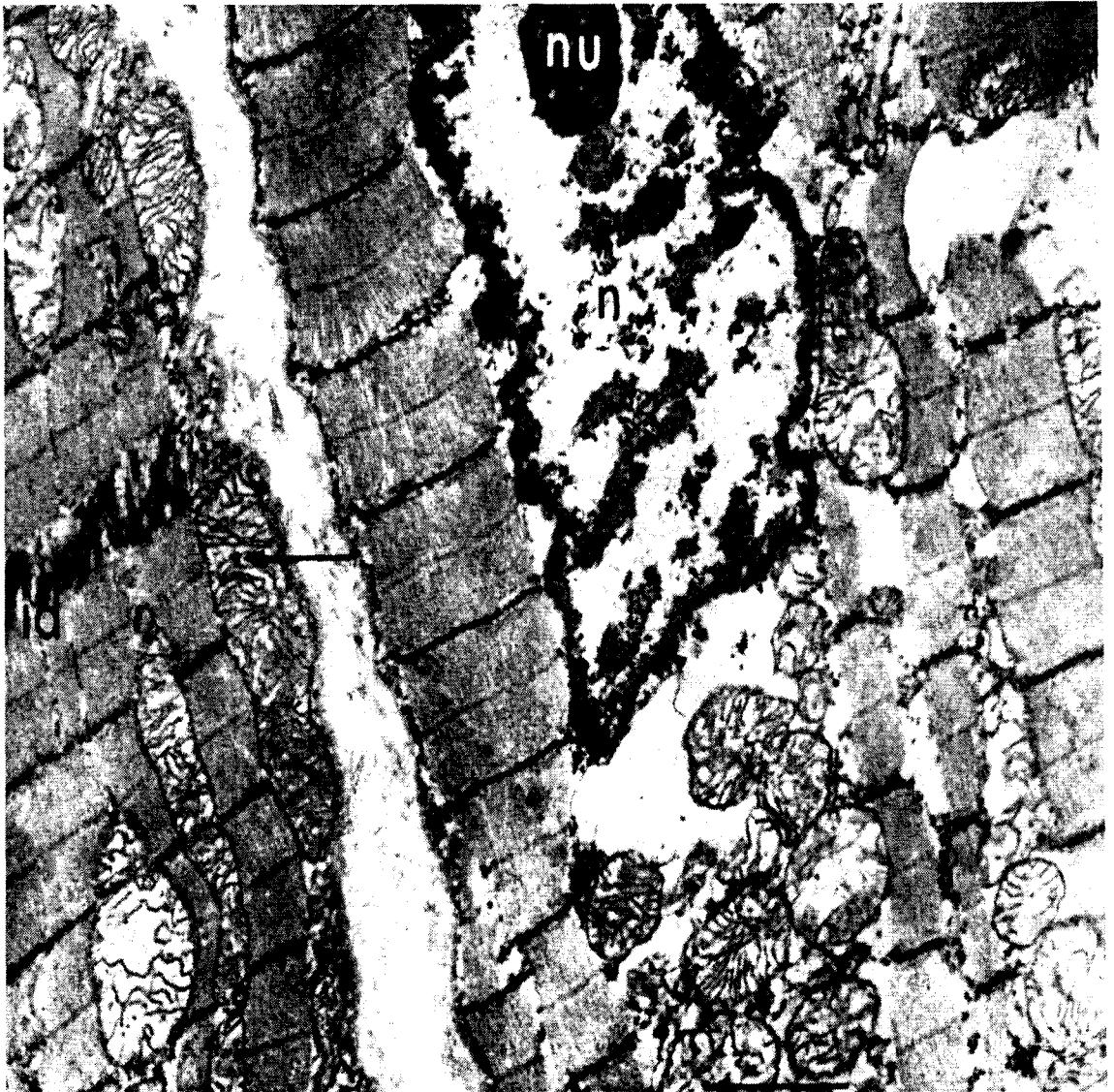


Figure 4.5

Forty minutes of ischemia. Chromatin margination in the nucleus (n) is more striking and mitochondrial changes are more advanced. Small granules are present in some mitochondria (arrow). Glycogen is virtually absent. Nucleolus (nu) and intercalated disc (id) visible.

RB Jennings Arch Pathol 1965: 79: 135 - 143



Figure 4.6

Sixty minutes of ischemia. The myofibrils are relaxed and clearly show an N line and the I band on either side of the Z band. The M band is present within the H band. Margination of nuclear chromatin is prominent and mitochondrial changes include loss of dense matrices, disruption of cristae and the presence of intramitochondrial granules.

(Lamping and Gross 1995, Przyklenk and Kloner 1988, Warltier 1988, Przyklenk 1989, Taylor 1990, Ehring 1992, Gross and Pieper 1992). However, in most of these studies it is unclear whether the beneficial effects reflected a direct protective action of the drug on the myocardium, or were mediated by favorable modifications of afterload, preload, heart rate and regional myocardial blood flow. If the drug is administered directly to the ischemic myocardium, the dose may be adjusted in such a way that no systemic hemodynamic alterations take place, so that any improvement in function is due to a direct myocardial protective action of the drug.

It is also of importance that therapeutic concentrations of the drug are present in the ischemic myocardium at the very onset of reperfusion. Drugs given intravenously during ischemia may not reach the ischemic area in the absence of a collateral circulation. Even in the presence of collaterals, a large intravenous dose is usually needed to achieve significant drug concentrations in the ischemic area. Systemic effects of the drug may then induce negative hemodynamic side effects. In contrast, local drug administration, such as antegrade intracoronary administration, has the advantage that the compound is selectively delivered into the targeted region, reaching a sufficient level without affecting the non-ischemic myocardium (Wang 2002). For these reasons in this study, drug or placebo were delivered directly into the proximal LAD, just distal to the occlusion. In addition, during coronary bypass procedures, there exists the unique opportunity to deliver a protective drug directly into the region at risk. This was one of the potential clinical applications of this study.

4.2.5 Timing of drug administration.

It is important that therapeutic concentrations of verapamil are *present* in the ischemic myocardium at the very onset of reperfusion (Wang 2002). Studies have shown that calcium channel blocking drugs effectively attenuate the reperfusion injury when administered **before** the onset of ischemia (Knabb 1986, Garcia-Dorado 1987, Vatner 1988). However, since long acting calcium antagonists were used, these studies do not distinguish between protection against ischemic injury and reperfusion injury.

A number of studies have shown that calcium antagonists administered immediately before or at the onset of reperfusion are protective (Klein 1989, Hatori 1993). Herzog (1997) showed that a low dose of diltiazem administered via the LAD in the swine heart during early reperfusion only, diminished **infarct size**. Because most studies have shown that calcium channel blockers provide benefit when given before ischemia or before reperfusion, but not at or after reperfusion, it has been postulated (Taylor 1990) that the benefit is due to protection from the effects of ischemia rather than from decreases in reperfusion related calcium influx. However bearing in mind that calcium influx and contraction band necrosis take place in the first few seconds of reperfusion, and that time is required for drug binding and action, it follows that the calcium channel blocker needs to be present, bound and active at the time of reperfusion to be effective. In the present study the verapamil infusion was started either 3

minutes before reperfusion or at the onset of ischemia i.e. 15 minutes before reperfusion.

4.2.6 The dosage of verapamil.

The usual clinical dose of verapamil in the human is 0.07 mg/kg (Opie 1988). This dose will result in systemic hemodynamic changes in most cases. The dose response curve of verapamil in the porcine model is unknown.

Intracoronary verapamil was used in two previous studies: Klein (1989), used intracoronary verapamil in a porcine model: After 45 minutes of ischemia intracoronary verapamil was started 60 seconds before reperfusion, continued at a high dose: 0.125 mg/min for 16 minutes and then a low dose: 0.062 mg/min for 30 minutes. Thus the total dose was $2\text{mg} + 1.86\text{ mg} = 3.86\text{mg}$ over 46 minutes, and the dose in the first 8 minutes was 1mg.

Lo (1985) used intracoronary verapamil in a canine model where dogs were subjected to 3 hours of coronary artery occlusion followed by 3 hours of reperfusion. In their protocol A, verapamil was infused at 0,01mg/kg/min from 90 minutes of occlusion until 1 hour after reperfusion. In their protocol B verapamil was infused at the same rate starting just before reperfusion and continuing throughout the reperfusion period. The total dose of verapamil in dogs weighing $\pm 30\text{kg}$ was: 45mg over 2½ hours. The dose in the first 8 minutes of infusion was 2.4mg.

In this study the average weight of the animal was $30.55 \pm 3.55\text{kg}$ and the average weight of the ischemic segment of the left ventricle that would be perfused by the intracoronary verapamil was $41.97 \pm 11.74\text{g}$. The **low dose** of verapamil chosen in this study was $0,5\text{mg}$ or $0,017\text{mg/kg}$. This dose is unlikely to cause any systemic hemodynamic changes in the animal. However a dose of $0,5\text{mg}$ of verapamil administered into the proximal LAD supplying $\pm 40\text{g}$ of myocardium, is a dose of $12,5\text{mg/kg}$ into the LAD perfusion bed. This dose may have local effects on the cardiomyocyte.

To decide on the “high” dose of verapamil to be used in this study, 6 pigs were subjected to an 8 minute systemic infusion of verapamil at 1ml/min in increasing concentrations (increasing total dosages). The **high dose** of verapamil was the highest dosage that, when administered in an infusion at 1ml/min for 8 minutes, did not lead to a decrease in mean arterial pressure of more than 10%. The dose so identified was 2mg .

2mg verapamil: $0,07\text{mg/kg}$ total weight
 20 mg/kg heart muscle
 50mg/kg ischemic heart muscle

In the present study, when 2 mg of verapamil was infused over 8 minutes into the normal LAD coronary artery (absence of ischemia), the mean arterial pressure decreased ($83 \pm 18.33\text{mmHg}$ to $66.3 \pm 10.7\text{mmHg}$), remained decreased at

10 minutes after the infusion ($69.5 \pm 11.8\text{mmHg}$), but had returned to baseline by 30 minutes after the infusion ($79.8 \pm 13.4\text{mmHg}$). However when 2mg of verapamil was infused into the occluded LAD coronary artery (presence of ischemia), either at the onset of ischemia, or 3 minutes before reperfusion, there was no change in mean arterial pressure.

4.2.7 The anesthetic technique.

The use of anesthesia is unavoidable in the open chest preparations. It is important to realize that these agents may modify the effects of myocardial ischemia and reperfusion, and affect cardiovascular responses to pharmacologic agents. In this study the anesthetic used was pentobarbital and fentanyl infusions, with pancuronium as the neuromuscular blocking agent. Ketamine was used as premedication.

Racemic ketamine has been found to block early and late preconditioning in rabbit and rat myocardium (Ko 1997, Zaugg 2003, Müllenheim 2001).

In the porcine model, pentobarbital reduces stroke volume by direct cardiac depression. It also increases systemic vascular resistance and heart rate. The drug however depresses the baroreceptor induced reflex tachycardia as the mean arterial pressure falls, and since hypotension is a common feature during myocardial ischemia, the ischemia induced response may be modified (Verdouw

1988). Most commonly used barbiturates inhibit mitochondrial K_{ATP} channel activity and therefore inhibit preconditioning (Zaugg 2003, Kohro 2001).

Fentanyl, through interaction with delta-opiate receptors, enhances mitochondrial K_{ATP} channel activity and results in myocardial preconditioning (Wild 1991, Zaugg 2002).

Pancuronium, which is the most widely used neuromuscular blocking agent in the swine model, has no effect on myocardial substrate utilization (Dhusmana 1982).

Inhalation agents, which profoundly influence myocardial functional and metabolic responses to ischemia and reperfusion in swine and dogs (Coezee 1993, Warltier 1988, Verdouw 1988, Merin 1982), and are potent agonists of myocardial preconditioning (Zaugg 2003), were avoided in the present study.

In view of the potential effects on ischemia and reperfusion, of the individual anesthetic agents used, an identical anesthetic technique was used in all animals in this study. Therefore any difference in function on reperfusion between the groups would be due to the different protocols for intracoronary verapamil or saline.

4.3 Evidence of myocardial ischemia.

Prior to examining the effects of the reperfusion injury it is important to establish that significant ischemia was indeed present in the experimental preparation.

This will be done in two parts:

- Functional evidence of myocardial ischemia.
- Metabolic evidence of myocardial ischemia.

4.3.1 Functional evidence of myocardial ischemia (Table 3.8.1).

Sudden occlusion of the LAD coronary artery caused severe regional myocardial dysfunction, evident within 3 to 4 beats in the LAD perfusion bed. The myocardial function in the region supplied by the circumflex coronary artery remained unchanged. Myocardial dysfunction preceded ST segment changes on ECG by several minutes.

The myocardial dysfunction was visible as weaker contraction followed promptly by systolic outward movement (bulging) of the affected ventricular wall. These classic visible signs of left ventricular segmental ischemia have been published by several investigators (Tennant and Wiggers 1935, Edwards and Rankin 1981, Forrester 1972, Perzada 1975, Glower 1988).

The following measured parameters changed significantly during ischemia compared to control values in all the groups studied:

1. There was an increase in maximum LAD segment length (L_{max}).
2. There was an increase in LAD segment length when the extrapolated left ventricular pressure is zero (L_0).
3. There was a decrease in both systolic shortening of the LAD segment (LAD syst dL) and LAD segment systolic shortening as a percentage of maximum LAD segment length (syst dL %).
4. There was an increase in both LAD segment post systolic shortening (PSS) and LAD segment post systolic shortening as a percentage of total LAD segment shortening.
5. There was a decrease in LAD segment stoke work (SW).

In this study the pressure length loop was used to analyze segment function. The pressure-length loop provides a sensitive way of examining the synchrony of contraction of a given segment with respect to the rest of the left ventricle (Wyatt 1975). Regional segment length reflects the time course of contraction of the ischemic segment interacting with the normal myocardium adjacent to it. Left ventricular pressure reflects the time course of contraction of the entire ventricle (Wyatt 1975).

After occlusion of the LAD coronary artery, segment lengthening instead of shortening occurs during systole in the ischemic segment of the LAD

perfusion bed (Tyberg 1974). This represents an inability of the ischemic muscle segment to contract against the stress developed by the non-ischemic LV muscle mass. The ischemic segment bulges outwards as the rest of the ventricle contracts. On the pressure-length loop segment length increases as a result of increased total LV pressure (Normally segment shortening results in an increase in LV pressure) (Fig. 4.7).

Under non-ischemic conditions, as the segment shortens during systole it produces an anti-clockwise loop or positive loop (Wyatt 1975). The area enclosed by a pressure-length loop over one cardiac cycle represents the mechanical work done by that segment during that cycle (Forrester 1974). Due to ischemia the affected segment is paralyzed and lengthens instead of shortening during systole, as it is "bulged" outward by the normally contracting non-ischemic ventricle. This ischemic paralysis results in a clockwise pressure-length loop, with a negative value for loop area. This indicates that work was done **by** the rest of the ventricle **on** the ischemic segment, as opposed to work done by the ischemic segment (Wyatt 1975). This systolic bulging explains the finding in the present study of an increase in maximum LAD segment length during ischemia. The ischemic paralysis described above also explains the decrease in systolic shortening of the LAD segment during systole seen in the present study and the clockwise direction of the pressure-length loop with the negative value for loop area explains the decrease in LAD segment strokework observed in the present study.

In this study acute ischemia shifted the pressure-length loop, and thus the end-systolic pressure-length relation (ESPLR) to the right. L_0 , which is the intercept of the ESPLR with the length (x) axes was therefore also right shifted. Hence, any particular pressure at the end of systole was associated with an increased regional end-systolic myocardial length. This increase in L_0 indicates a decreased elasticity at the end of systole which underscores the previously noted paralysis of the acutely ischemic myocardium. These findings confirm previously reported results (Tyberg 1974, Osakada 1983, Theroux 1974, Warltier 1988, Ross 1976, Victory 1991).

As shown by Stahl (1986), the slope of the ESPLR in the present study remains constant during ischemia. This does not represent an active process, and is simply the result of an interaction between the increase in LV pressure and the absence of regional shortening (Victory 1991, Sunagawa 1982). Elasticity (contractility) certainly does decrease during ischemia and this index loses its validity during acute ischemia.

Concerning systolic function during acute ischemia the consistent finding among investigators is a right shifted pressure-length relationship and the complete loss of systolic shortening (Sunagawa 1983, Kaseda 1984).

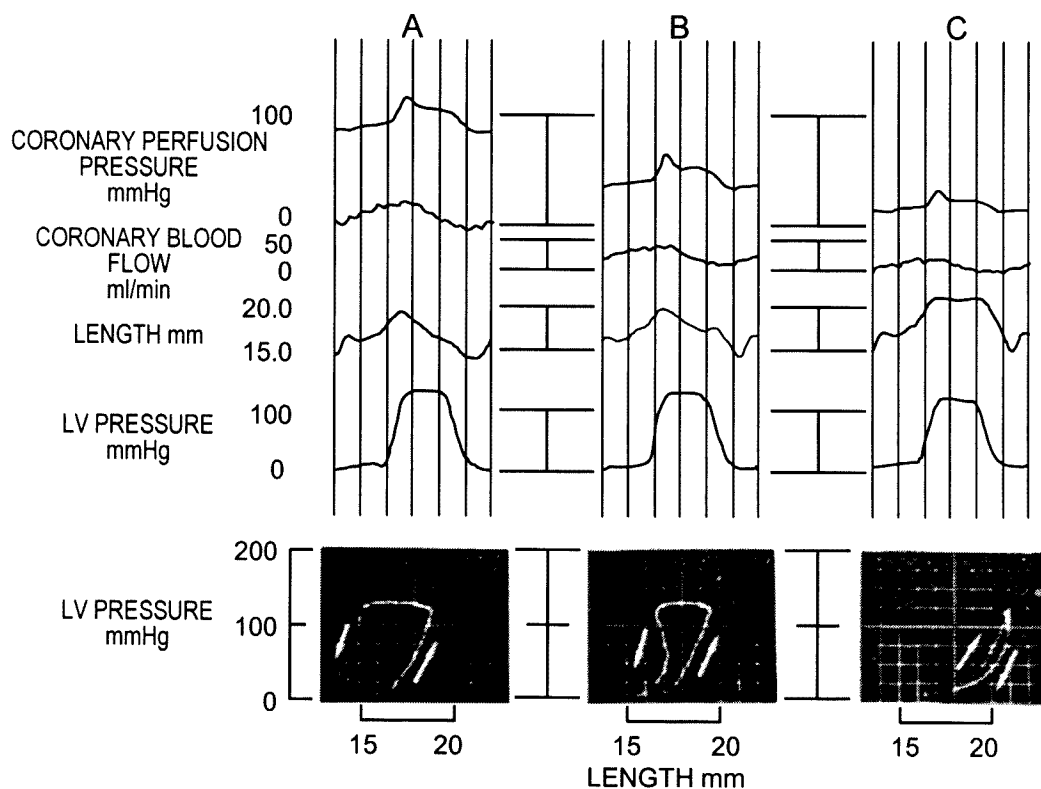


Figure 4.7

Effect of graded coronary flow reduction on coronary blood flow, regional epicardial segment length, aortic and LV pressure and the relationship between the epicardial segment length and LV pressure.

A: Normal contraction.

B: Decreased systolic shortening.

C: Systolic dyskinesis in the ischemic segment.

Post-systolic shortening (PSS), which is an indication of early diastolic dysfunction (Bonow 1981, Theroux 1976, Takayama 1988), is the systolic shortening that occurs after aortic valve closure, it is represented by the upper left hand corner of the pressure-length loop (Brown 1987). PSS is also expressed as a percentage of total shortening (PSS%) (Brown 1987, Coetzee 1991). The significant increase in PSS and PSS as a percentage of total shortening seen in this study during ischemia, confirm the results of several investigators (Theroux 1974, Vatner 1977, Smalling 1983, Wyatt 1975, Forrester 1976, Kumada 1979, Brown 1987). Coetzee (1991) showed that in the open chest animal model PSS% greater than 20% indicates ischemia. In the present study the average PSS% during ischemia was 50%.

In this study there is therefore significant functional evidence of myocardial ischemia.

4.3.2 Metabolic evidence of myocardial ischemia (Table 3.8.2).

In this study blood samples were taken from the proximal aorta for arterial blood gas measurements and from the anterior inter ventricular vein for venous blood gas measurements. The anterior inter ventricular vein runs parallel to the LAD which was occluded during the ischemic section of the protocol. Whereas coronary sinus blood represents mixed venous blood from both ischemic and non ischemic myocardium (Owen 1970), venous blood sampled from the anterior

inter ventricular vein accurately reflects venous drainage originating almost exclusively from the myocardium perfused by the LAD under normal and ischemic conditions (Vinten-Johansen 1987, Constantini 1975, Roberts 1976, Marshall 1974). Furthermore reperfusion after transient ischemia does not change the unique origin of the anterior inter ventricular vein blood. This technique may therefore be used to reliably assess oxygen utilization and metabolic variables in the myocardium perfused by the LAD (Vinten-Johansen 1987). Also, validation of the selective sampling of anterior inter ventricular venous effluent in ischemic-reperfused segments, makes reliable serial assessment of the metabolic status of this zone possible, *independently* of other areas in the in vitro heart (Vinten-Johansen 1987) (Fig. 4.8).

The results of venous blood gas samples taken 10 minutes after LAD coronary artery occlusion during the ischemic step of the protocol differed significantly from control values in all groups studied.

When the LAD coronary artery was occluded, there was, as expected, a significant reduction in both CBF ($1,58 \pm 5,59$ ml/min vs $27,13 \pm 7,78$ ml/min) and CBF/100 g ($4,08 \pm 14,27$ ml/min vs $73,76 \pm 26,54$ ml/min) compared to control measurements. The reduction in CBF is similar to that in other studies (Heyndrickx 1975, Laxson 1989, Owen 1970). As a result of the decrease in CBF there was a significant reduction in oxygen consumption in the ischemic segment compared to control values ($0,17 \pm 0,46$ ml/min vs $2,47 \pm 0,85$ ml/min). This is confirmed by other studies (Laxson 1989, Liedtke 1978).

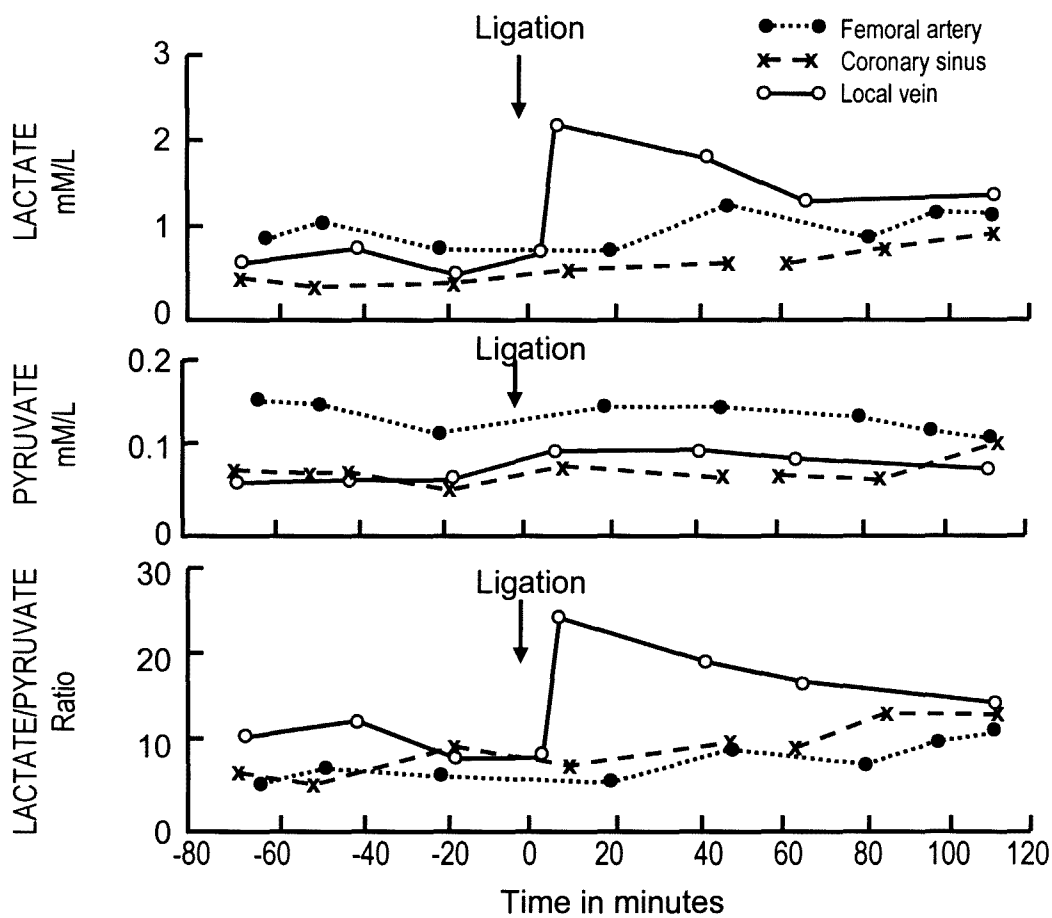


Figure 4.8

Lactate, pyruvate and the ratio lactate/pyruvate in arterial, local venous and coronary sinus blood before and after coronary artery occlusion.

The accumulation of CO_2 within the myocardium reflects the balance between tissue CO_2 production and its clearance by regional blood flow (Khuri 1975). During ischemia, coronary blood flow and its associated delivery of oxygen, are drastically reduced. As a result myocardial cells convert to anaerobic metabolism which causes intracellular acidosis (Liedtke 1976, Scheuer 1967) due to the production of lactic acid (Liedtke 1976). The lactic acid diffuses into the interstitium resulting in an increased concentration of hydrogen ions. These combine with HCO_3^- , resulting in the production of CO_2 and water (Khuri 1979). Any continued aerobic metabolism within the cells also produces carbon dioxide, which diffuses into the interstitium, as CO_2 is freely diffusible across cell membranes (Khuri 1979). As ischemia becomes more severe, there is an increase in the production of hydrogen ions and, as a result in the quantity of CO_2 that is produced. Simultaneously there is a reduction of CO_2 clearance. Consequently the severity of myocardial ischemia is reflected in the magnitude of the elevation of intramural (interstitial) PCO_2 (Khuri 1979). Khuri (1979) confirmed that the magnitude of rise of intramural PCO_2 , following coronary artery occlusion, is an excellent predictor of the extent of myocardial ischemic injury, as assessed histologically, as well as with the reduction of regional myocardial blood flow. The rise in intramural PCO_2 is also a sensitive indicator of the presence of myocardial ischemia.

Hillis (1979) showed that interventions that modify the severity of ischemia can be evaluated by measuring intramural carbon dioxide tension. The rapid

diffusability of CO₂ through tissues results in an essentially equal PCO₂ in interstitial tissue and coronary venous blood. Wallace and Hastings (1942) suggested that it was unlikely that any gradient in CO₂ tension of significant magnitude existed between tissue and serum due to the high value of the diffusion coefficient for CO₂. This was confirmed by Case (1979), who concluded that extracellular myocardial PCO₂ is essentially equal to that in coronary venous blood.

In the present study there was a statistically significant increase in PCO₂ in the anterior inter ventricular vein blood during the ischemic step of the protocol compared to control values (9,79 ± 1,77 kPa vs 7,78 ± 2,67 kPa). This suggests the presence of significant ischemia.

The arterio-coronary venous lactate difference is a commonly used indicator of transmural metabolic change (Gonschior 1992). During aerobic activity the cardiac muscle utilizes lactate for energy production. However, when oxygen is not in adequate supply, the cardiomyocyte produces lactate by anaerobic glycolysis (Gibbs 1978, Waters 1978) (Fig. 4.9 and 4.10).

It is commonly considered a sign of ischemia when the extraction of lactate out of the arterial blood is less than 10% (under normal non-ischemic conditions it is 20 to 40%) and lactate production begins (Waters 1978). Arterio-coronary lactate difference is negative if coronary venous lactate concentrations are higher than arterial lactate concentrations, and this implies a net production of

lactate. It has been shown that coronary venous lactate concentration during a moderate stenosis is highest 10 to 20 minutes after the onset of stenosis (Ferrari 1986). In the present study measurements were made after 10 minutes of ischemia.

There is a close and linear correlation between the degree of ischemia and the magnitude of changes in arterio-venous lactate difference (Gonschior 1992). Hence, lactate production is a distinct, easily measurable metabolic abnormality that can be quantitated in myocardial ischemia (Scheuer 1967). Coronary venous lactate concentration is also a sensitive marker of ischemia, and will increase before the appearance of clinical symptoms of ischemia (Guth 1990).

Guth (1990) showed that increased lactate release is a sensitive marker of myocardial ischemia in patients and experimental animal models. He demonstrated a close inverse relation between regional myocardial lactate release and regional subendocardial blood flow during graded ischemia.

In this study there was an increase in anterior interventricular venous lactate levels during ischemia compared to control values ($2,97 \pm 0,21 \text{ mmol.l}^{-1}$ vs $0,57 \pm 0,71 \text{ mmol.l}^{-1}$). There also was a significant change in the arterio-venous lactate difference as a percentage of arterial lactate concentration between the ischemic step and the control values: $-1465 \pm 35,88\%$ during ischemia vs. $-51,1 \pm 8,84\%$ control values. The above results confirm that significant ischemia was indeed present during the ischemic step of this protocol.

Due to the increased hydrogen ion production during ischemia there was an expected significant decrease in anterior intra ventricular venous pH during ischemia ($7,24 \pm 0,02$ vs $7,34 \pm 0,08$). There was also a decrease in bicarbonate concentration in the venous blood during ischemia due to increased consumption of this buffer ($30,51 \pm 0,14$ mmol.l⁻¹ during ischemia compared to $33,48 \pm 1,2$ mmol.l⁻¹ before ischemia).

In the present study there was a significant decrease in anterior intra ventricular vein PO₂ during ischemia compared to control values ($2,81 \pm 0,8$ kPa vs $3,48 \pm 0,00$ kPa). This finding is confirmed by Gonschior (1992) in a study on the effects of acute LAD coronary artery occlusion in pigs. Analysis of coronary venous blood samples in Gonschior's study revealed that PO₂, O₂ content and oxygen saturation significantly decreased, whereas lactate significantly increased during stenosis. In clinical studies, Messer (1962) found increased myocardial oxygen extraction after exercise and a decline in coronary sinus oxygen content in 82% of patients with coronary artery disease. Coronary venous PO₂ is considered a sensitive indicator of myocardial oxygenation because it represents the end capillary PO₂ (Feigl 1983). However, although there is a decrease in PO₂ during myocardial ischemia, studies have shown that changes in intramural oxygen tension with coronary artery occlusion are unreliable in the quantification of the severity of ischemia.

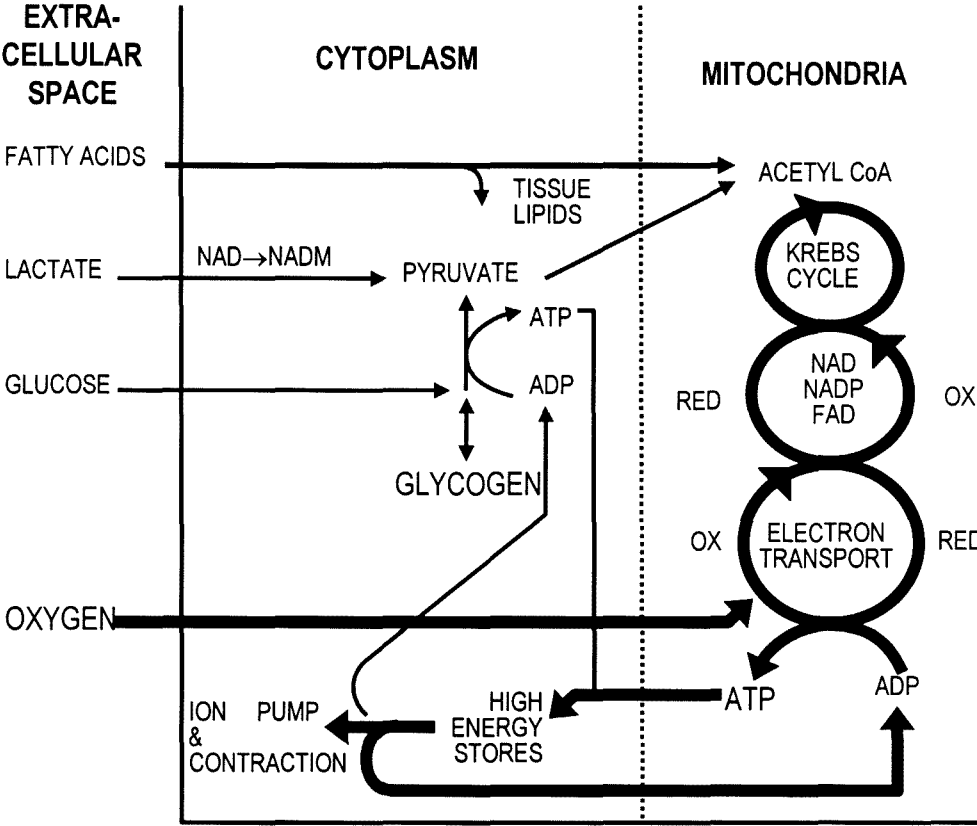


Figure 4.9

Energy pathways in the normal myocardium. In the presence of sufficient oxygen, rates of electron transfer and oxidative phosphorylation is governed by the supply of ADP.

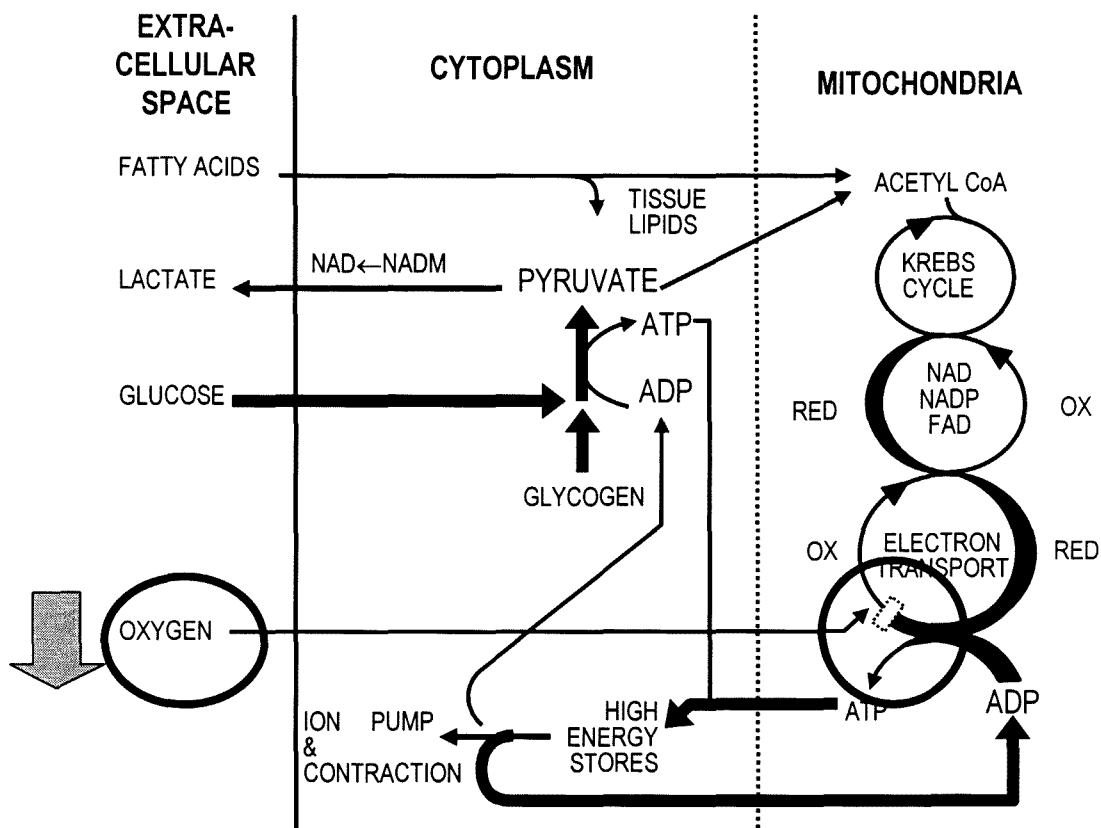


Figure 4.10

Energy pathways in the myocardium in the presence of hypoxia. There is a relative block of electron transport and oxidative phosphorylation is inhibited. High energy phosphate stores decline. Degradation of acetyl CoA cannot proceed and the use of glucose and glycogen increases and pyruvate accumulates.

This metabolic evidence confirms that significant ischemia was indeed present in the experimental model used in this study.

4.4 Evidence of reperfusion (Table 3.8.2).

In all animal studied CBF decreased during ischemia and was increased at 10 minutes after reperfusion compared to both control, and other post ischemia values. By 30 minutes after reperfusion, CBF had returned to control values and remained so until the end of the experiment. These findings confirm those of other investigators (Laxon 1989, Lange 1984, Stahl 1986, Heyndrickx 1978), and provide evidence that the previously ischemic myocardium was indeed reperfused.

4.5 The characteristic reperfusion injury.

The effects of a 15 minute regional coronary artery occlusion, followed by reperfusion on:

- Myocardial function
- Regional myocardial bloodflow
- Myocardial histology

were examined and described by Heyndrickx et al. in 1978. These effects are remarkably similar in all respects to the findings obtained in the two saline groups in the present study, and are discussed below.

Effects of coronary occlusion and reperfusion on the myocardial function:

Overall left ventricular function:

Heyndrickx found that a 15 minute regional coronary occlusion induced only slight changes in global left ventricular function: (Fig. 4.11).

- Left ventricular systolic pressure remained unchanged.
- dP/dt decreased.
- Heart rate increased slightly.

By 15 minutes after reperfusion all values had returned to normal and remained so.

The finding in the two saline groups in this study confirmed the above with no change in left ventricular systolic pressure, dP/dt , or heart rate.

Regional wall thickness

Regional wall thickening occurs during systole when myocardial fibers shorten. Changes in wall thickness are thus reciprocal to changes in segment length. Segment shortening is directly proportional to wall thickening. In the present study segment length and segment shortening were measured as indexes of systolic function.

Heyndrickx demonstrated a severe loss of systolic wall thickening during coronary artery occlusion. Upon reperfusion systolic wall thickening increased transiently to a level slightly below pre-occlusion baseline. By 15 minutes after reperfusion however, systolic wall thickening was again decreased and remained so for as long as three hours (Fig. 4.12).

In the present study, in the saline groups, as reported in the Heyndrickx study, segment shortening decreased during ischemia and remained decreased for the duration of the experiment.

Heyndrickx found that not only was the wall thickening depressed, but it also showed a marked delay in "time-to-peak" shortening. This resulted in a marked change in the configuration of the phasic wave form for wall thickening, with wall thickening (or segment shortening) persisting into early diastole. This shortening in early diastole is referred to in the present study as post systolic shortening. In the Heyndrickx study, post systolic shortening normalized only after three hours of reperfusion.

In the present study post systolic shortening increased during ischemia in both saline groups. In the one saline group baseline levels had again been reached by 60 minutes after reperfusion, but in the other saline group post systolic shortening was still significantly increased at the end of the experiment (90 minutes after reperfusion).

Heyndrickx, in his study found that end diastolic wall thickness also decreased during ischemia but to a lesser degree than systolic wall thickness. (In the present study end diastolic wall thickness is represented by the segment length value L_{max} , the reciprocal of wall thickness). On reperfusion there was an immediate rebound of diastolic wall thickness to above control values, however by 15 minutes post reperfusion, end diastolic wall thickness was no different from control.

In the saline groups in this study, L_{max} did increase during ischemia, only to return to control values by 30 minutes after reperfusion.

Hence, despite the use of saline as an infusion in the placebo (control) groups in this study, the course of the reperfusion injury closely followed that described by previous investigators (Heyndrickx 1978, Smith 1980, Lamping 1985, Przyklenk 1986, Gross 1986).

Effects of coronary artery occlusion and reperfusion on regional myocardial blood flow:

Heyndrickx (1978) found an expected decrease in bloodflow during ischemia, followed by a hyperemic response on reperfusion. By 15 minutes after reperfusion myocardial bloodflow had returned to pre-ischemic control values.

This finding corresponds to the finding in both saline groups in this study where coronary bloodflow decreased during ischemia (intentionally decreased with LAD occlusion), was increased (twice baseline levels) at 10 minutes post reperfusion and had returned to control values by 30 minutes post reperfusion.

Effects of a 15 minute coronary occlusion and reperfusion on myocardial histology:

Histologic and histochemical studies after a short period of occlusion did not show any anatomical or histological defects (Heyndrickx 1978). This finding fits the concept of “stunning” which, by definition, implies a temporary decrement in function which is totally reversible. In the current study, histology was not done and cannot be compared to the already accepted findings.

Conclusion:

In the present study the author attempted to study the effects of verapamil on the elements of the reperfusion injury i.e. stunning and arrhythmias. Data from the saline groups in this study (no active intervention) confirms that the model closely follows the pattern of temporary dysfunction after reperfusion already published and serves to validate the experimental model used.

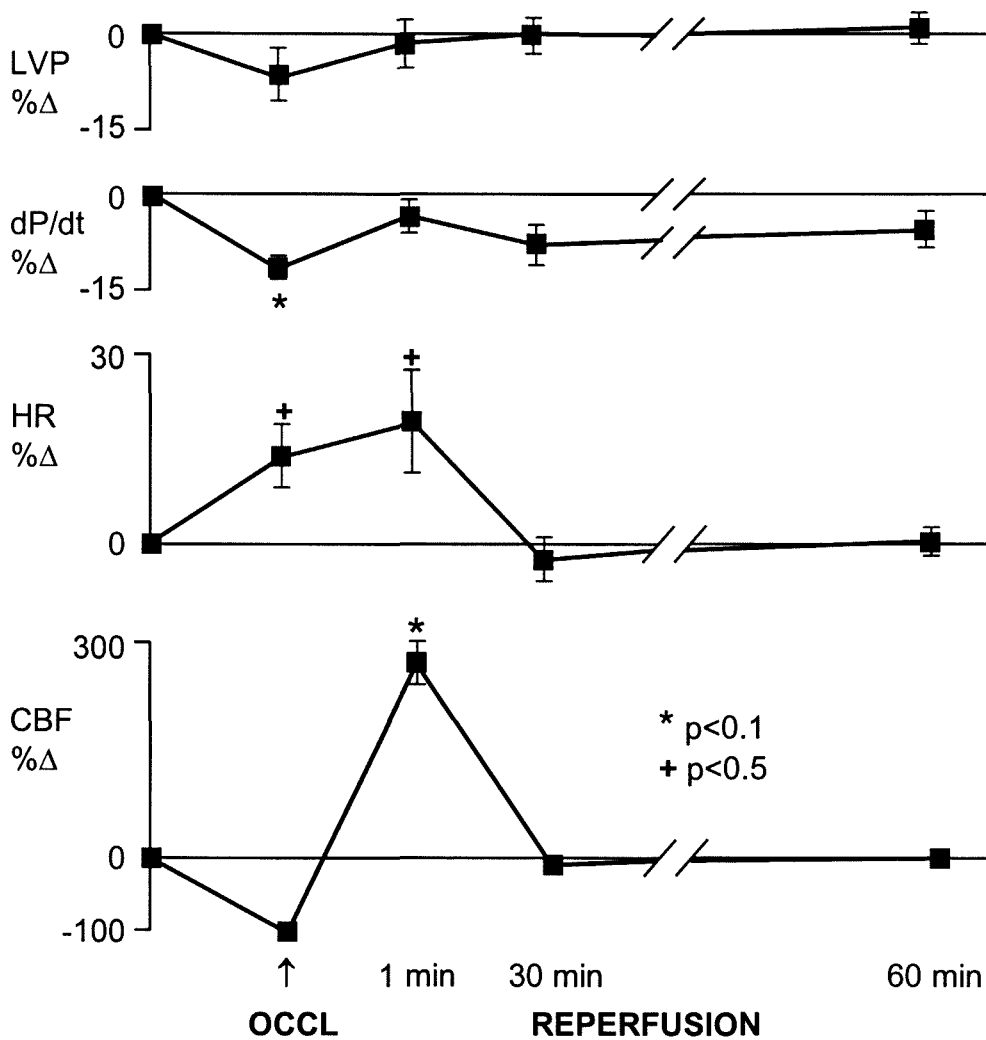


Figure 4.11

Changes in left ventricular pressure (LVP) , dP/dT, heart rate (HR) and coronary blood flow (CBF) during coronary artery occlusion and reperfusion. At 30 – 60 minutes after reperfusion was initiated no significant effects could be demonstrated on left ventricular function (LV) and CBF.

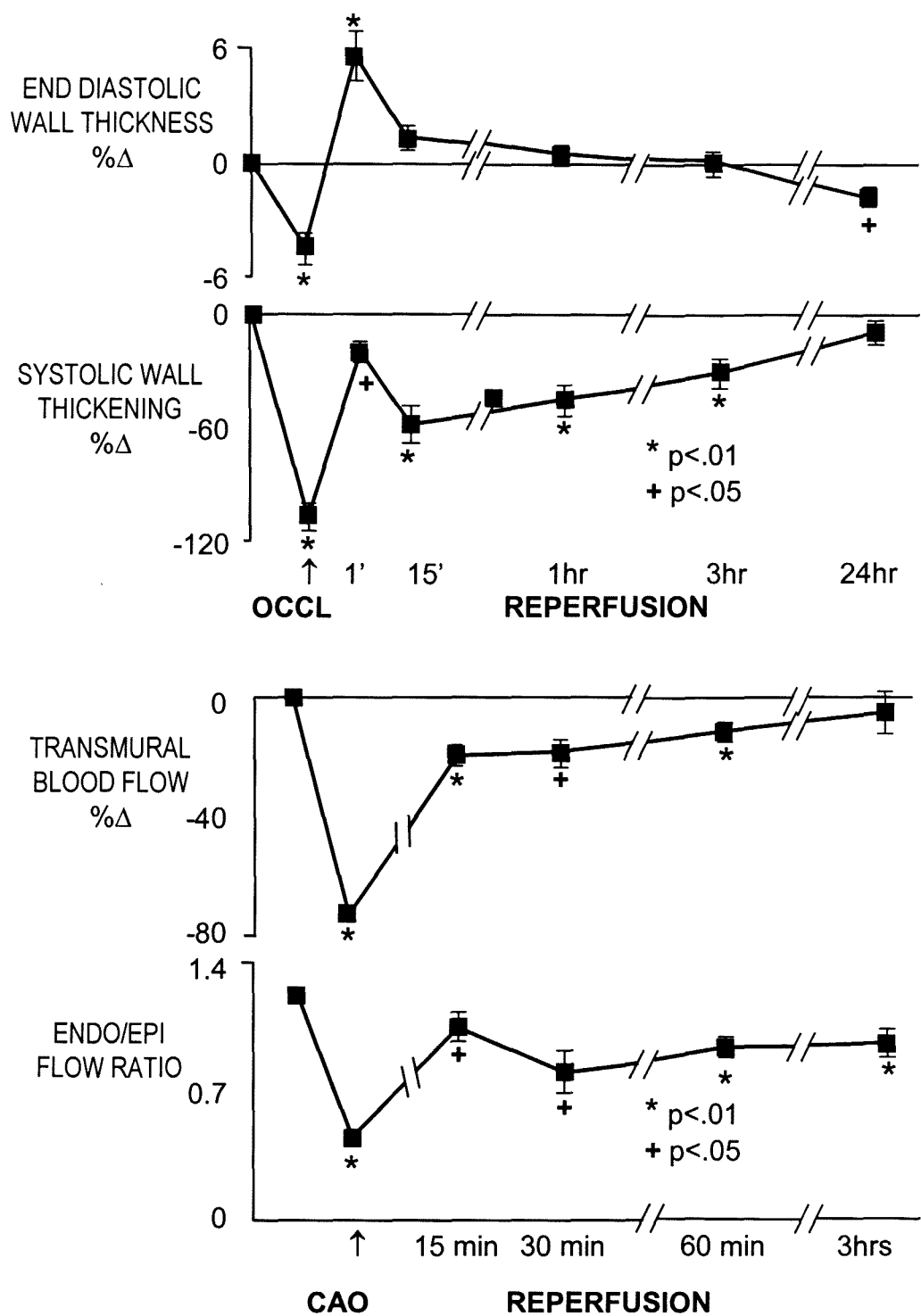


Figure 4.12

Effect of 15 minute occlusion and reperfusion on end-diastolic wall thickness, systolic wall thickening (top graph) , transmural coronary blood flow and endo and epicardial flow ratio (lower graph). Values are average / SEM.

G Heyndrickx Am J Physiol 1978; 234: H653 – H659

4.6 Verapamil and the reperfusion injury.

The role of calcium and the calcium antagonists in the reperfusion injury was elegantly described by Bourdillon (1982).

“Myocardial ischemia in humans occurs after myocardial infarction or during open heart surgery under conditions of cardiopulmonary bypass. Limitation of the size of the necrotic area in myocardial infarction and preservation of myocardial function after cardiac surgery are important therapeutic objectives. During reperfusion after a period of ischemia, calcium accumulates in myocardium and particularly in mitochondria.

Since part of the total calcium influx into the myocardial cell is through the slow calcium channel of the cardiac action potential, this channel may be the pathway for calcium accumulation associated with reperfusion after ischemia. Verapamil and other calcium antagonists block the slow calcium current of the action potential. When these drugs are given prior to or at the onset of a period of ischemia, they have been shown to preserve tissue ultra structure, to reduce enzyme release, to diminish S-T elevation, to improve hemodynamic function, to maintain tissue ATP, and to prevent calcium accumulation in whole tissue and isolated mitochondria. The uniformly beneficial results obtained in such experiments are in sharp contrast to the results of experiments in which calcium antagonists have been administered, not prior to ischemia, but either after the ischemic myocardium has ceased to contract or at the time of reperfusion. Under

these conditions, cardiac function is not preserved, tissue ATP is not maintained, calcium accumulation is not prevented, and enzyme release is unaltered.

Calcium accumulation on reperfusion has been shown to be due to increased influx probably related to a specific abnormality of ionic channels. The extent of calcium accumulation is related to the severity and duration of ischemia, and to the degree of mechanical recovery."

Several in vitro studies support the finding that when calcium antagonists are given prior to a period of myocardial ischemia, tissue damage is prevented or delayed, ATP and mitochondrial function are preserved, calcium accumulation is reduced and functional recovery is improved (Henry 1977, Watts 1980, Lefer 1979, Nayler 1980, Nayler 1987, Hamm 1983, Bourdillon 1982, Nayler 1990, Yao 1994, Boraso 1993, Kirkels 1992, Bush 1982, Buser 1991, Nayler 1976, Yamamoto 1983, Watts 1986).

In contrast to the studies by Kirkels (1992), Higgins (1982) and later studies by Massoudy (1995) and Bernstein (1996), Watts (1986) found that calcium antagonists had to depress pre-ischemic contractile function in order to afford protection.

Most in vitro studies of ischemia-reperfusion failed to demonstrate a beneficial effect of calcium channel blockers administered after the onset of ischemia or on reperfusion (Watts 1980, Nayler 1978, Bourdillon 1982, Ferrari 1986, Heusch

1992, Watts 1986, Yamamoto 1983). In Bourdillon's study (1982) this negative finding was not due to insufficient time for verapamil binding and effect before reperfusion, because the introduction of verapamil during low flow ischemia, 10 minutes before reperfusion, also failed to reduce calcium uptake. Nayler's study (1978) also used a hypoxic model where verapamil was added to the perfusate after the decline of developed tension.

These in vitro studies suggest that pre-treatment with calcium antagonists is beneficial to the *ischemic* myocardium due to a cardioplegic effect. Myocardial metabolism and $\dot{V}O_2$ are decreased (Nayler 1972, Fleckenstein 1983, De Jong 1985, Nayler 1990). The rate of ATP consumption during ischemia is reduced (Higgins 1983, Bourdillon 1982, Jolly 1981, Nayler 1980, Watts 1980, Weishaar 1980). Sufficient ATP is maintained to ensure preservation of membrane function and intracellular calcium homeostasis (Ferrari 1984, Bourdillon 1982, Nayler 1980). As membrane function is preserved, the rate of sodium gain and potassium loss will be slowed (Nayler 1980) and therefore the consequent calcium gain and resultant ischemic contracture will be decreased. Because intracellular calcium accumulation is decreased, the mitochondria will be protected against calcium overload (Bernstein 1996, Ashraf 1984). Carafoli (1985) found that mitochondria that are overloaded with calcium deteriorate functionally and have impaired ATP production. Hence "protected" mitochondria will be more functionally intact during reperfusion, and better able to support ATP synthesis.

The ATP sparing effect of calcium antagonists is only present if the drug is administered before the onset of ischemia, and absent in hearts treated only during reperfusion (Watts 1980, Watts 1985, Watts 1986). As most studies (De Jong 1985, Watts 1986) showed that calcium antagonists decreased lactate production by the ischemic myocardium, the increased ATP levels in the hearts protected by calcium antagonists are not due to increased glycolytic flux. The increased ATP levels are most likely due to reduced energy consumption by the ischemic myocardium in the presence of calcium antagonists (Watts 1986). Interestingly, contractile depression during early ischemia does not account for all the ATP sparing effects of, or the decrease of glycolysis due to the calcium antagonists. Watts (1986) found that diltiazem administered to hearts that had been rendered quiescent with potassium arrest prior to the onset of ischemia, significantly reduced lactate accumulation. Therefore the effects of diltiazem on lactate production, or the need for glycolysis due to ATP-depletion is at least partly independent of the contractile effects of the drug.

The following in vitro studies do however demonstrate a protective affect of calcium antagonists administered after the onset of ischemia or at reperfusion.

Bernstein (1996) in isolated isovolumic blood perfused rabbit hearts subjected to 90 minutes of low flow ischemia (CBF 22 – 24% of baseline) followed by 30 minutes of reperfusion, found that felodipine was protective if administered from 15 minutes *after* the onset of ischemia until 15 minutes after reperfusion. Bernstein found that felodipine conferred a significant protection against

ischemic diastolic dysfunction (less ischemic contracture), and on reperfusion as there was significantly better recovery of systolic and diastolic function in the presence of felodipine. However, even though contractility, $\dot{V}O_2$, lactate production and coronary bloodflow were identical between the two groups during ischemia, ATP levels in the felodipine group were more than twice that in the control group. This suggests that the protection afforded by felodipine was at least in part, due to an ATP preserving effect during ischemia. The fact that there was significantly less severe acidosis during ischemia in the felodipine group (a finding supported by Kirkels, 1992), suggests that less anaerobic glycolysis was necessary in this group, probably due to better ATP preservation and a lower ATP requirement.

Massoudi (1995) in isolated working guinea pig hearts subjected to 15 minutes of ischemia followed by 15 minutes of reperfusion found that short term post-ischemic application of the calcium antagonists gallopamil was as effective in restoring pump function as pre-ischemic application. In addition gallopamil did not need to depress pre-ischemic cardiac function in order to elicit protection.

Higgins (1984) studied the effects of verapamil, nifedipine and diltiazem in isolated working rat hearts subjected to 30 minutes of ischemia followed by 30 minutes of reperfusion. All the drugs improved recovery of function and inhibited intracellular calcium accumulation if administered before ischemia. If given only during reperfusion nifedipine was almost as effective as when administered before ischemia, but verapamil and diltiazem were not effective.

Van Amsterdam (1990) suggested two modes of protection against ischemia-reperfusion by the calcium antagonists: A negative inotropic or energy sparing activity protecting during ischemia, and vasodilatation resulting in improved recovery during reperfusion. However, Higgins (1982) suggested that the improved perfusion seen during reperfusion in calcium antagonist treated hearts may result from **prevention of ischemic contracture** (via a cardio protective effect), which preserves a better transmural distribution of myocardial perfusion during the ischemic period and during reperfusion, rather than from direct coronary vasodilatation by the drug.

Results obtained in these buffer perfused, non working, globally ischemic hearts may not necessarily be extrapolated to regional ischemia in blood perfused hearts in intact animals.

Confirming the findings in the in vitro studies Warltier (1988) and Lamping (1985) reported a beneficial effect on cardiac functioning on reperfusion after pre treatment with calcium antagonists before a short period of reversible ischemia in the intact canine model. However, in all these studies the use of calcium antagonists caused a statistically significant decrease in mean arterial pressure, and this may have contributed to the beneficial effect.

Ehring (1992) studied the effects of nisoldipine administered at different times, in attenuating the reperfusion injury after a 15 minute coronary artery occlusion

in anesthetized open chest dogs. In this study the mean arterial pressure was kept constant with an aortic balloon pump and regional myocardial blood flow during ischemia and reperfusion was the same in all groups. They reported that nisoldipine was beneficial only if given before occlusion, but not when given 5 minutes before reperfusion or 4 minutes after reperfusion. Nisoldipine was administered intravenously in these animals and it is possible that the nisoldipine administered during ischemia did *not reach* the affected myocardium, and that this may be the reason why no beneficial effect was seen in the group where nisoldipine was administered 5 minutes before reperfusion. When nisoldipine was administered 4 minutes after reperfusion, the transient calcium overload seen immediately after reflow (Kusuoka 1987) had abated and cytosolic calcium levels had probably already returned to normal (Steenbergen 1987).

Functional recovery in this study was relatively poor; only 60% of control value was achieved in the group pretreated with nisoldipine, and there was no recovery in the other groups. This is unusual, as in most studies of myocardial stunning, even the control groups recover 30 – 40% of pre-ischemic function after an extended period of reperfusion. This finding may be due to the anesthetic technique used (Thiamylal 15mg/kg ivi; 1,4% enflurane in 2:1 nitrous oxide/oxygen mixture).

In the canine model of myocardial stunning Przyklenk (1988) reported that an intravenous verapamil infusion started 30 minutes before a 15 minute artery occlusion and continued for 180 minutes of reperfusion (total dose 2,45 mg/kg)

completely ablated contractile dysfunction (115% of pre-ischemic values) and preserved ATP content in the post ischemic myocardium. However, verapamil infusion started directly before reperfusion, and even started 30 minutes after reperfusion (same total dose as pre-treatment) resulted in recovery of contractile function to 66% of control values compared to only 31% if no verapamil was administered. ATP content was however *not* preserved in these animals. The conclusion in this study was that verapamil administered at or after reperfusion significantly enhanced contractile function, and pre-treatment with verapamil prevented myocardial stunning.

A very large dose of verapamil (2.45 mg/kg) was administered systemically in this study, and as can be expected, this had an effect on systemic hemodynamics, leading to a decrease in MAP, heart rate, and inotropy from the onset of the infusion, hence more favorable hemodynamics before and during ischemia in the pre-treated group. This large dose of verapamil before and especially during ischemia would have increased coronary collateral circulation to the ischemic area (due to vasodilatation and reduced ischemic contracture) in the pre-treated group in this canine model, and this may have decreased the severity of ischemia. There was also insufficient time for drug binding and effect of the verapamil administered at the onset of reperfusion, for it to be effective during the critical first moments of reperfusion when increased calcium flux is relevant (Kusuoka 1987, Steenbergen 1987).

In contrast to the present study, Przyklenk found that verapamil administered at the time of reperfusion did not protect against ventricular fibrillation. Only verapamil pre-treatment was protective. This again is probably due to insufficient time for drug binding and effect at the very onset of reperfusion.

Przyklenk (1989) in open chest anesthetized dogs subjected to 15 minutes of coronary artery occlusion followed by reperfusion, compared the effects of high dose *intravenous* nifedipine administered 30 minutes after reperfusion to low dose *intracoronary* nifedipine administered 30 minutes after reperfusion. They reported that the high dose intravenous nifedipine restored contractile function of the stunned myocardium to pre-occlusion values, however it also caused a decrease in afterload and an increase in regional myocardial blood flow. Low dose intracoronary nifedipine did not alter afterload or coronary blood flow, but did restore systolic contractile function to 90% of pre-occlusion values (compared to 37% if no nifedipine was given). These results indicated that the improvement in systolic contractile function was not entirely the result of decreased afterload and increased myocardial blood flow, and that nifedipine may act by favorably modulating calcium flux within the stunned myocytes.

The confounding factor in this study is that the calcium antagonist was only administered *30 minutes after* the onset of reperfusion in all animals. As argued earlier myocardial stunning results from a transient calcium overload immediately after reflow (Kusoaka 1987), and the increased cytosolic calcium levels returned to normal within a few minutes after reperfusion (Steenbergen 1987, Marban

1987). By 30 minutes after reperfusion the “damage is done” and intracellular calcium has returned to normal levels. This is not the critical period of reperfusion.

Park (1996) investigated the effect of intravenous nisoldipine in the conscious porcine model of repetitive ischemia. Pigs underwent 10 two minute coronary artery occlusions separated by two minutes of reperfusion, in the treatment group, nisoldipine was administered from 15 minutes before the first occlusion until 30 minutes after the last reperfusion. The systemic hemodynamics remained unchanged, there was zero collateral blood flow in the ischemic region, the ischemic myocardial function was identical in treatment and control groups and importantly, nisoldipine infusion did not affect myocardial **contractility** in the non-ischemic zone. This study demonstrated that nisoldipine markedly attenuated myocardial stunning independently of any hemodynamic changes or changes in coronary blood flow. This suggests a direct cardio protective action of nisoldipine.

There was active preconditioning in this study, but this was present in the control and nisoldipine groups, and nisoldipine conferred added protection. Interestingly, the recovery of function in the nisoldipine group in this study (58% of pre-ischemic value by 60 minutes post reperfusion, 66.8% by 120 minutes) was slower than in the author’s study (100% of pre-ischemic values by 10 minutes after reperfusion). There was also a convergence of recovery between the nisoldipine group and the control group throughout reperfusion. By 5 hours

after reperfusion the nisoldipine group had recovered to 97.9% of pre-ischemic values and the control group tot 88.9% of pre-ischemic values.

Park proposed that the major mechanism of action of nisoldipine in this study was to decrease calcium influx during ischemia, resulting in an attenuation of ischemic injury, and as a secondary effect, the attenuation of the reperfusion injury. However, as argued earlier (Chapter 1) the increase in cytosolic calcium during ischemia is the result of **ATP depletion**, failure of sarcolemma and sarcoplasmic reticulum calcium ATP-ase, failure of Na⁺/K⁺ATP-ase resulting in intracellular sodium accumulation and reversal of the Na⁺Ca²⁺ exchanger. The L-type calcium channels affected by nisoldipine do not play a role in the increase in cytosolic calcium during ischemia, but are important at the very onset of reperfusion. Nisoldipine does however probably have an ATP sparing effect **during ischemia** (Watts 1980, 1985, 1986).

Verdouw (1983) studied the effects of intravenous nefidipine administered from 10 minutes before reperfusion until 5 minutes after reperfusion in the anesthetized open chest porcine model, subjected to 30 minutes of low flow ischemia (25 to 30% of normal flow), followed by 60 minutes of reperfusion. This study reported 75% recovery of systolic and diastolic wall thickening during reperfusion in the nisoldipine group, compared to only 50% recovery in the control group.

As the calcium antagonist in this study was administered timeously (10 minutes before reperfusion, and low flow ischemia as opposed to zero flow ischemia was used, there may well have been time for local drug binding and effect by the time of reperfusion. This study provides evidence of a protective effect of calcium antagonists at the time of reperfusion.

There have been several studies (Klein 1989, Gao 2001, Hatori 1993, Segawa 2000, Gourine 2001, Segawa 2002) where intracoronary administration of calcium antagonists has been effective in attenuating the ischemia-reperfusion injury in the open chest porcine model. However, all these studies had LAD occlusion times of 45 minutes or longer, resulting in infarction as opposed to reversible myocardial stunning.

The hypothesis of this study was that an adequate dose of verapamil, administered timeously directly into the coronary artery supplying the ischemic segment, would attenuate the reperfusion injury due to a direct effect on the myocardium.

To evaluate myocardial function indexes of:

- ***Systolic function***
- ***Diastolic function***
- ***Myocardial metabolism***

were considered.

The indexes of ***systolic function*** measured were:

- LAD L max (maximum LAD segment length)
- LAD syst dL (the systolic shortening of the LAD segment)
- LAD syst dL% (LAD segment systolic shortening as a percentage of maximum LAD segment length)

The latter parameter was used because the magnitude of change in segment length depends in part on the initial segment length (placement of crystals), the changes expressed in absolute length (mm) would vary in relation to the extent that the initial segment lengths varied. Therefore segment shortening observed in this study was also calculated as a percentage change of initial segment length. LAD segment stroke work was also considered as an index of function (Chapter 2).

The indexes of ***diastolic function*** used were:

- Post systolic shortening (PSS)
- Post systolic shortening as a percentage of total segment shortening (PSS%) (Coetzee 1991)

The hypothesis of this study was confirmed in that both groups of animals where 2mg of verapamil was administered either at the onset of ischemia (group 5) or 3 minutes before reperfusion (group 3), had faster recovery of systolic and

diastolic myocardial function compared to the groups where saline or the low dose of verapamil were administered.

The greatest difference was in the indexes of ***systolic function***. In the saline groups and the low dose verapamil group, these indexes had not yet recovered to control values by the end of the experiment at 90 minutes post reperfusion (Table 3.2.3, 3.4.3, 3.6.3) whereas the group that received 2mg of verapamil at the onset of ischemia had recovered full systolic function (all indexes normalized) by the first measurement at 10 minutes post reperfusion (Table 3.5.3). In the group where 2mg of verapamil was administered from 3 minutes before reperfusion, all the indexes of systolic function had normalized by the second measurement at 30 minutes post reperfusion (Table 3.3.3).

The recovery of ***diastolic dysfunction*** was more varied between groups. The groups receiving 2mg of verapamil either from 3 minutes before reperfusion or from the onset of ischemia, had full recovery of diastolic dysfunction by the first measurements at 10 minutes after reperfusion (Table 3.3.3, 3.5.3). The low dose verapamil group had recovered diastolic function by 30 minutes after reperfusion (Table 3.2.3), the group where saline was administered from the onset of ischemia, by 60 minutes after reperfusion, and the group that received the saline infusion starting 3 minutes before reperfusion, had not recovered diastolic function by the end of the experiment (Table 3.6.3, 3.4.3). The recovery of diastolic function is more graded between groups compared to the

recovery of systolic function. The former may be a more sensitive marker of the degree of stunning.

The effects of reperfusion on diastolic function as shown in this study, are similar to those reported by Krause (1986). They reported a decrease in activity of the sarcoplasmic reticulum ATP-ase following brief ischemic episodes. This resulted in the decreased ability to transport calcium during relaxation and hence prolonged contraction into the diastolic period (contracture). Weiss (1986) found that delayed contractile recovery during reperfusion is attributable in part to an adverse effect of increased diastolic calcium oscillation induced by calcium overload during *reperfusion*.

Although the high dose verapamil groups performed better than both the saline groups and the low dose verapamil group, there were important differences between the two high dose groups. The group where the verapamil infusion was started at the onset of ischemia (group 5), had full recovery of *systolic* function by the first measurement at 10 minutes after reperfusion. The group where the verapamil infusion was started 3 minutes before reperfusion (group 3) had slower recovery of systolic function (30 minutes after reperfusion).

The degree of ischemia in both high dose verapamil groups was similar, as evidence by venous lactate increases. Also VO_2 /100g and VO_2 /100g/beat were the same in the two groups, decreasing during ischemia and normalizing by 10 minutes after reperfusion. Coronary blood flow also decreased to a similar

degree during ischemia, was increased to a similar degree at 10 minute after reperfusion, and had normalized to control values by 30 minutes after reperfusion in both groups.

There are however **four** possible explanations for the difference in performance between the two high dose verapamil groups:

Firstly verapamil is protective against ischemia per se. Verapamil administered prior to ischemia has consistently been shown to attenuate the depletion of high energy phosphate stores both in models of global ischemia and reperfusion (Watts 1986, Nayler 1980, Neely 1984) and in in vivo canine models of stunning (Lange 1984, Przyklenk 1988). This ATP preserving effect was however **not** seen in in vitro models of global ischemia where verapamil was administered after the onset of ischemia (Bersohn 1983, Nayler 1987), or in in vivo models of regional ischemia when verapamil was administered at or after reperfusion (Przyklenk 1988). As ATP levels were not measured in this study they cannot be compared, however the literature strongly supports an ATP sparing effect of verapamil if present during ischemia.

Secondly the faster recovery of function in the group where verapamil was administered from the onset of ischemia may be due to the fact that a higher dose of verapamil had been administered by the critical point where reperfusion took place. The entire 2mg had been infused, and had time for binding and action before reperfusion occurred. In the group where the verapamil infusion

was started 3 minutes before reperfusion only 0.75 mg of verapamil had been administered by the onset of reperfusion. As discussed previously, it is the calcium influx at the onset of reperfusion which sets in motion the cascade of events leading to contraction band necrosis (Piper 2003, Piper 2004). The more L-type calcium channels that are blocked at this crucial time, the less severe the reperfusion injury (Chapter 1).

Thirdly, there was a longer period of wash-out of ischemic metabolites, specifically lactate, in the group where verapamil was administered from the onset of ischemia. In this group anoxic flow was present for the first 8 minutes followed by 7 minutes of ischemia. In the group where the verapamil infusion was started 3 minutes before reperfusion, ischemia was present for 12 minutes followed by a 3 minute period of anoxic flow. Neely (1984) demonstrated that if anoxic low flow was present, the ability to recover ventricular function was maintained for a longer exposure to ischemia, and occurred at much lower levels of ATP compared to zero coronary flow. These observations suggests that accumulated metabolic products play an important role in myocardial injury. This role is independent of oxygen supply and ATP-levels. In Neely's study there was a strong negative correlation between tissue levels of lactate during ischemia and recovery of function on reperfusion. In the present study the longer period of anoxic flow present in the group where verapamil was started from the onset of ischemia may have contributed to the faster recovery of contractile function. This may also explain the difference in recovery of diastolic function in the two saline (control) groups in this study. Diastolic dysfunction recovered more

quickly in the group where the saline infusion was started at the onset of ischemia (anoxia) compared to when it was initiated 3 minutes before reperfusion (ischemia). Although this difference between the effects of hypoxia and ischemia, may partly explain the differences in recovery between the two high dose verapamil groups, and between the two saline groups, the significantly faster recovery of both systolic and diastolic function in both ischemic and anoxic high dose verapamil groups compared to the anoxic saline group is due to the presence of verapamil.

Fourthly, verapamil depresses contractility only when coronary blood flow is normal (Romson 1986) as was the case during the last 5 minutes of verapamil infusion in the group where the infusion was started 3 minutes before the onset of reperfusion therefore in this group verapamil had a negative inotropic effect which was absent in the group where the verapamil infusion was started at the onset of ischemia.

Several investigators have suggested that the stunned myocardium may be sensitive to both arterial vasodilatation (Stahl 1986, Lamping 1985, Przyklenk 1988, Warltier 1988, Tayler 1990), and increases in regional myocardial blood flow (Stahl 1986, Berdeaux 1976, Matsuzaki 1984, Heusch 1987). Improvement in systolic contractile function would result from the inotropic effect of baroreflex-mediated sympathetic activation due to systemic vasodilatation, or from the increase in myocardial blood flow. **Neither of these two factors are applicable in the present study:**

As a porcine model with a sparse coronary collateral circulation was used, and the LAD coronary artery was completely occluded during ischemia, there probably was no difference in tissue blood flow during ischemia between the groups. In addition data from this study showed no difference in the degree of ischemia. On reperfusion there was a similar hyperemic response in coronary blood flow, with values at 10 minutes post reperfusion being higher than control values, but coronary blood flow had normalized to control values in all groups by 30 minutes after reperfusion. Coronary blood flow did not differ between the different groups.

Secondly the improved function was not due to more favorable systemic hemodynamics during ischemia or reperfusion, as mean arterial pressure did not decrease at any time compared to control values in any of the groups. Heart rate also remained constant throughout and left ventricular end-systolic pressure and volume did not decrease or differ. Therefore the improved function seen on reperfusion in the two high dose verapamil groups is due to a *direct effect* of verapamil on the myocardium.

Investigators using models of both global (Krause 1984), and regional ischemia (Krause 1986) have shown that calcium flux within myocytes is impaired after brief ischemic episodes, and that elevated concentrations of intracellular calcium adversely affect both mitochondrial ATP production (Nayler 1987) and myocardial contractility (Shen 1981).

To further clarify this direct effect of verapamil on the myocardium, the effect of an intracoronary infusion of 2mg of verapamil on the non-ischemic myocardium was studied.

In contrast to the ischemic model, the mean arterial pressure decreased when verapamil was infused in the absence of myocardial ischemia. Mean arterial pressure remained decreased 10 minutes after the infusion and had only normalized by 30 minutes after the infusion. The possible explanation for the decrease in mean arterial pressure is that the markedly increased coronary blood flow seen after infusion of verapamil in the non-ischemic heart, caused a faster washout of verapamil from the myocardium and increased systemic effects. Alternatively, the hypotension may have been the result of negative inotropic effects of verapamil. Romson (1986) found that the effects of verapamil varied as a function of myocardial blood flow: During normal coronary blood flows, verapamil had a negative inotropic effect while at low flow (30% of normal), it had no effect on contractile function.

Verapamil did seem to have a negative inotropic effect in the this non-ischemic model, as maximum LAD segment length did increase significantly after the verapamil infusion, and control values were only reached by 30 minutes after the infusion was terminated. Although the other indexes of systolic function i.e. segment shortening, decreased numerically after the infusion, the reduction did not reach statistical significance. The decrease in systolic function may have

been masked in part, by a decreased afterload, due to the fall in mean arterial pressure.

Verapamil infusion also resulted in diastolic dysfunction. Although PSS was not nearly as severe as that seen during ischemia, it did increase directly after the verapamil infusion and had returned to control values by 30 minutes after the infusion (Table 3.1.3). This differential effect of verapamil on diastolic function in the normal and ischemic hearts has been well described (Pagel 1993). Verapamil enhances regional diastolic mechanics in the ischemic heart (Bonow 1981) but , in the normal heart, calcium channel blockers impair isovolemic relaxation (Walsh 1985).

This decreased inotropy and lusitropy in the normal heart is readily explained by the decrease in calcium influx through the voltage gated L-type calcium channels resulting in decreased calcium induced calcium release from the sarcoplasmic reticulum, and consequently less calcium available for binding troponin C. However as stated earlier (Romson 1986) this negative inotropic effect of verapamil is absent if verapamil is administered during ischemia. This differential effect of verapamil on contractility in the presence or absence of ischemia, may also, in part, explain the slower recovery of contractile function in the group where the 2mg of verapamil infusion was started 3 minutes before reperfusion (compared to when it was started at the onset of ischemia), as a large portion of this infusion would have been delivered during reperfusion, when the coronary blood flow was raised. Verapamil may therefore have contributed

to the negative inotropic effect of the reperfusion injury, slightly delaying recovery of function in this group. No negative inotropic effect would have resulted from the 2mg of verapamil infused at the onset of ischemia, as this infusion was completed 7 minutes before reperfusion.

The pertinent finding in the non-ischemic group was that despite a threefold increase in LAD coronary artery blood flow on verapamil infusion, oxygen consumption in the LAD segment decreased significantly (1.34 ± 0.5 vs $2.48 \pm 1.6 \text{ mL} \cdot \text{min}^{-1}$). By 10 minutes after verapamil infusion oxygen consumption was again approaching control values ($1.76 \pm 0.45 \text{ mL} \cdot \text{min}^{-1}$), while coronary blood flow was still twice control values. Usually oxygen consumption in the myocardium determines coronary blood flow (Ross, 1985). In the presence of verapamil there seems to be an uncoupling of this relationship resulting in luxury perfusion. It is also important to note that this is not merely a normal $\dot{V}O_2$ in the face of coronary vasodilation due to verapamil; oxygen consumption is in fact clearly decreased (Table 3.1.1, 3.1.6). This is probably due to decreased myocardial metabolism and possibly due to decreased cardiac function (decreased external work). Nayler and Szeto (1972) were the first to describe the decrease in myocardial $\dot{V}O_2$ due to verapamil.

Nayler (1972) showed that verapamil decreased myocardial $\dot{V}O_2$ and improved myocardial efficiency. Myocardial $\dot{V}O_2$ is known to depend on heart rate (Evans and Matsuoka 1914, Cohn and Steele 1935), velocity of contraction (Sonnenblick 1965), and work done by the ventricle in overcoming pressure

(Evans and Matsuoka 1914, Katz and Feinberg 1958). Verapamil decreases the maximum tension developed, the rate at which tension is developed, the area under the contraction curve, and the heart rate (Nayler 1972). It alters neither the ability of the sarcoplasmic reticulum to accumulate calcium, or the activity of calcium ATP-ase (Nayler 1972). Verapamil does however reduce the amount of calcium which is stored at membrane located binding sites, hence the amount of calcium taken up by the cell per beat is decreased (Nayler 1972). Verapamil therefore also interferes with the mechanisms whereby intracellular distribution of calcium in cardiomyocytes is regulated: As calcium entry into the cell per beat is decreased, calcium release from the sarcoplasmic reticulum will be decreased, resulting in less calcium available for binding to Troponin C.

Verapamil may have two distinct effects during ischemia and reperfusion; one specific to ischemia, due to the effect of verapamil on the myocardial metabolism ($\dot{V}O_2$), and one specific to reperfusion due to the effect of verapamil on the L-type calcium channels on the sarcolemma, resulting in decreased calcium entry, and therefore decreased calcium induced calcium release from the sarcoplasmic reticulum at the onset of reperfusion (Barry 1993, Beukelmann 1988, Sperelakis 1988).

Verapamil decreases myocardial $\dot{V}O_2$ (or myocardial metabolism) therefore, during ischemia, it may result in a better supply: demand ratio due to decreased demand, resulting in “less severe” ischemia. “Less severe” ischemia means less intracellular calcium accumulation during ischemia and smaller sarcoplasmic

reticulum calcium stores and sarcoplasmic reticulum calcium release on reperfusion (Chapter 1).

Bourdillon (1982) and Watts (1985) suggested that a negative inotropic effect, resulting in decreased myocardial $\dot{V}O_2$, may be a mechanism by which calcium antagonists enhance post ischemic myocardial function. Smith (1976) suggested that verapamil may reduce myocardial injury through a selective depression of contractility and $\dot{V}O_2$ in ischemic myocardium, when given in a dose too low to affect contractility in normal myocardium. De Jong (1984) suggested that calcium antagonists directly reduced myocardial $\dot{V}O_2$ (not necessarily by negative inotropy), and so diminish the effect of flow impairment.

Decreased breakdown of ATP has been suggested to be a mechanism by which calcium channel blockers may protect ischemic myocardium, independently of their effect on calcium entry (De Jong 1984, Hamm 1983, Nayler 1980). Several investigators have demonstrated ATP preservation associated with pre-treatment with verapamil (Hamm 1983, Nayler 1980, Watts 1985), and diltiazem (De Jong 1984, Hamm 1983). Lange (1984) showed that if verapamil is infused from one hour before a 15 minute coronary artery occlusion, until the onset of reperfusion, endocardial ATP levels are preserved compared to an untreated group. Przyklenk (1988) also found that endocardial ATP stores were preserved when verapamil was administered 30 minutes before coronary artery occlusion. Verapamil given at or after reperfusion however, had no beneficial effect on depletion of ATP stores.

In Lange's study however, heart rate and mean arterial pressure was significantly lower in the treatment group throughout the experiment than in the control group. In the Przyklenk study mean arterial pressure was also decreased significantly by verapamil infusion throughout the experiment in the groups that received verapamil 30 minutes before occlusion (where ATP was preserved), compared to the groups receiving verapamil at or after reperfusion. This decrease in mean arterial pressure may result in lower ATP turnover, and may have contributed to the ability of verapamil to preserve ATP stores in the presence of ischemia. However, Nayler (1976) in *isolated* globally hypoxic rabbit hearts, observed that verapamil reduced myocardial uptake of sodium and loss of potassium, slowed the rate of creatine kinase release, decreased ATP and phosphocreatine breakdown, maintained the fine morphology of cells and prevented contraction bands.

The author's deduction is that if ATP is preserved during ischemia, membrane ATP-ase will continue to function, sodium and consequently calcium will not accumulate within the cell, the direction of the $\text{Na}^+\text{Ca}^{2+}$ exchanger will not reverse, hence the total intracellular calcium concentration during ischemia will be lower leading to lower sarcoplasmic reticulum calcium stores and less calcium induced calcium release on reperfusion, and attenuation of the reperfusion injury (Chapter 1).

Kloner (1982), in open chest dogs subjected to one hour of myocardial ischemia, observed that verapamil treatment resulted in striking preservation of the mitochondrial ultrastructure. Verapamil has also being shown to have a protective effect on the mitochondrial respiratory function (Clements 1978). Ischemia followed by reflow causes marked ventricular contracture (Apstein 1977, Bush 1982, Henry 1977, Jolly 1981, Weishaar 1980). This contracture is associated with large increases in mitochondrial calcium content (Henry 1977). Calcium antagonists protect mitochondria against calcium overload during ischemia (Bernstein 1996, Ashraf 1984), and prevent the deterioration of mitochondrial respiration (Boraso 1993). From the above observations it follows that verapamil treatment may preserve mitochondrial integrity and hence mitochondrial ATP synthesis. The balance between ATP synthesis and degradation may be better preserved in verapamil treated hearts:

- Increased ATP synthesis due to protection of the mitochondria against calcium overload.
- Decreased ATP requirement due to a lower myocardial metabolism ($\dot{V}O_2$) as seen in this study, and as shown by Nayler (1972).

Attenuation of ischemic acidosis by calcium antagonists (Kirkels 1992, Bernstein 1996) may also contribute to myocardial protection (Ochi 1988, Hara 1988, Ichiahra 1979). The higher pH during ischemia may be the result of reduced anaerobic glycolysis during ischemia (Kupriyanov 1988). Catecholamine

stimulation enhances the breakdown of glycogen (Ichihara 1979), and has several other detrimental effects during ischemia and reperfusion e.g. vasoconstriction, platelet aggregation, excessive energy utilization and arrhythmias. Calcium antagonists reduce norepinephrine stores before ischemia (Nayler 1987, Chaudhry 1984, Nayler 1988), and reduce norepinephrine release during ischemia and reperfusion (Nayler 1984, Nayler 1987, Nayler 1985). The calcium antagonists may therefore facilitate myocardial protection by indirectly increasing intracellular pH during ischemia.

Several studies have demonstrated that verapamil given **at the time of reperfusion** will enhance contractile function without preserving myocardial ATP content (Przyklenk 1988, Przyklenk 1986, Neely 1984). Kusuoka (1987) demonstrated that myocardial ATP after ischemia did not correlate with functional recovery. These studies provide evidence that the depletion of high energy phosphate stores alone cannot fully account for the contractile dysfunction seen after a transient brief period of myocardial ischemia. It therefore appears that there has to be a **second positive effect of verapamil occurring at the time of reperfusion**.

Neely (1984) demonstrated that the inability of a reperfused heart to recover mechanical function was not due solely to low levels of ATP. The two most important factors determining the ability of reperfused hearts to recover ventricular function were firstly, the level of extracellular calcium and secondly the accumulation of tissue lactate during ischemia. The effect of calcium on

ischemic damage was largely independent of residual ATP levels during reperfusion.

There is an abundance of evidence confirming massive influx of calcium and intracellular calcium overload upon reperfusion. Jennings (1985), Bourdillon (1982), Shen (1972), and Nayler (1981), all demonstrated a massive and uncontrolled influx of calcium into the cardiomyocyte upon reperfusion. Calcium entry upon reperfusion plays a major role in the pathogenesis of myocardial stunning (Kusuoka 1987). Mitchell (1992) showed that calcium influx occurs during the onset of reperfusion and contributes to stunning of the heart; and that sarcoplasmic reticulum calcium release on reperfusion also contributes to overall calcium overload.

Kitakaze (1987) noted that a brief period of transient global ischemia significantly elevated levels of free cytosolic calcium during both the final minutes of ischemia and the initial 10 minutes of reflow. Kusoaka (1987) showed that myocardial stunning results from the transient calcium overload immediately after reflow and Steenbergen (1987) and Marban (1987) confirmed that this increased cytosolic calcium level returned to normal within a few minutes after reperfusion.

There is also evidence for the role of calcium antagonists at the time of reperfusion. Ashraf (1984), Nayler (1980), and Higgins (1984) found that diltiazem decreases intracellular calcium accumulation upon reperfusion. Higgins

and Blackburn (1984) found that nifedipine given at the time of reflow was virtually as effective in restoring function as nifedipine given before ischemia in the isolated working rat heart model of myocardial stunning. Przyklenk (1988), Bush (1985), and Knabb (1986) also showed that calcium channel blockers are protective against stunning even when given before reperfusion. Bush (1985), in conscious dogs, showed that administration of diltiazem one hour after LAD occlusion prevents the worsening of segmental contractile dysfunction early after coronary reperfusion after two to four hours of LAD occlusion.

The following two facts are evident:

1. Increased intracellular calcium contributes to myocardial stunning and ventricular fibrillation (Opie 1989, Bolli 1990, Kloner 1989, Tani 1990, Duff 1995).
2. The reperfusion injury results from changes occurring both during ischemia and after reperfusion.

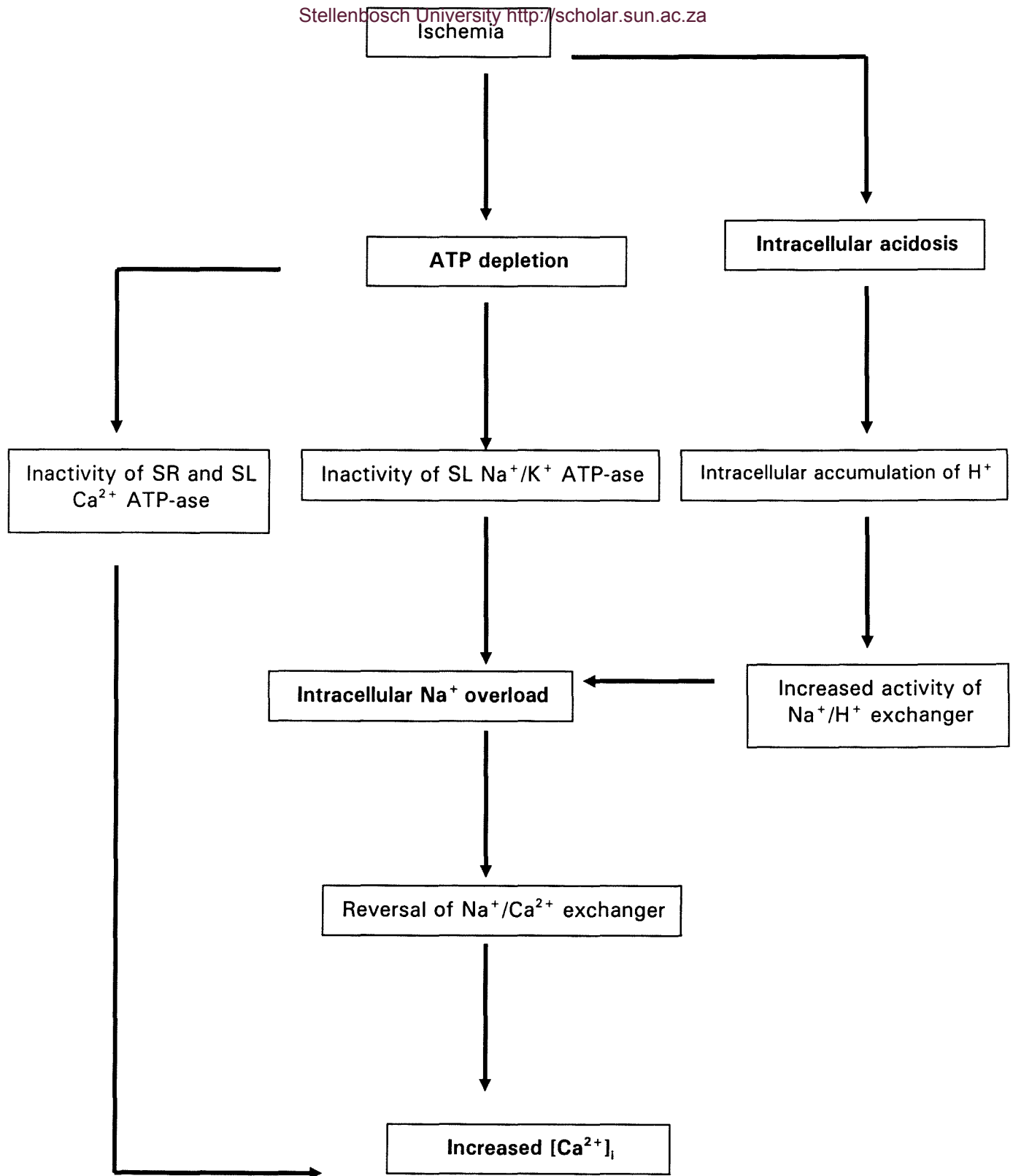
Intracellular calcium increases **during ischemia** due to:

- Depletion of ATP.
- Inactivity of sarcoplasmic reticulum Ca^{2+} ATP-ase.
- Inactivity of sarcolemmal Na^+/K^+ /ATP-ase.
- Intracellular acidosis.

- Increased activity of Na^+/H^+ exchanger.
- Intracellular Na^+ accumulation.
- Reversal of $\text{Na}^+/\text{Ca}^{2+}$ exchanger.
- Increased intracellular calcium ion concentration.

Tani (1990)

These processes can be summarized in a schematic fashion:



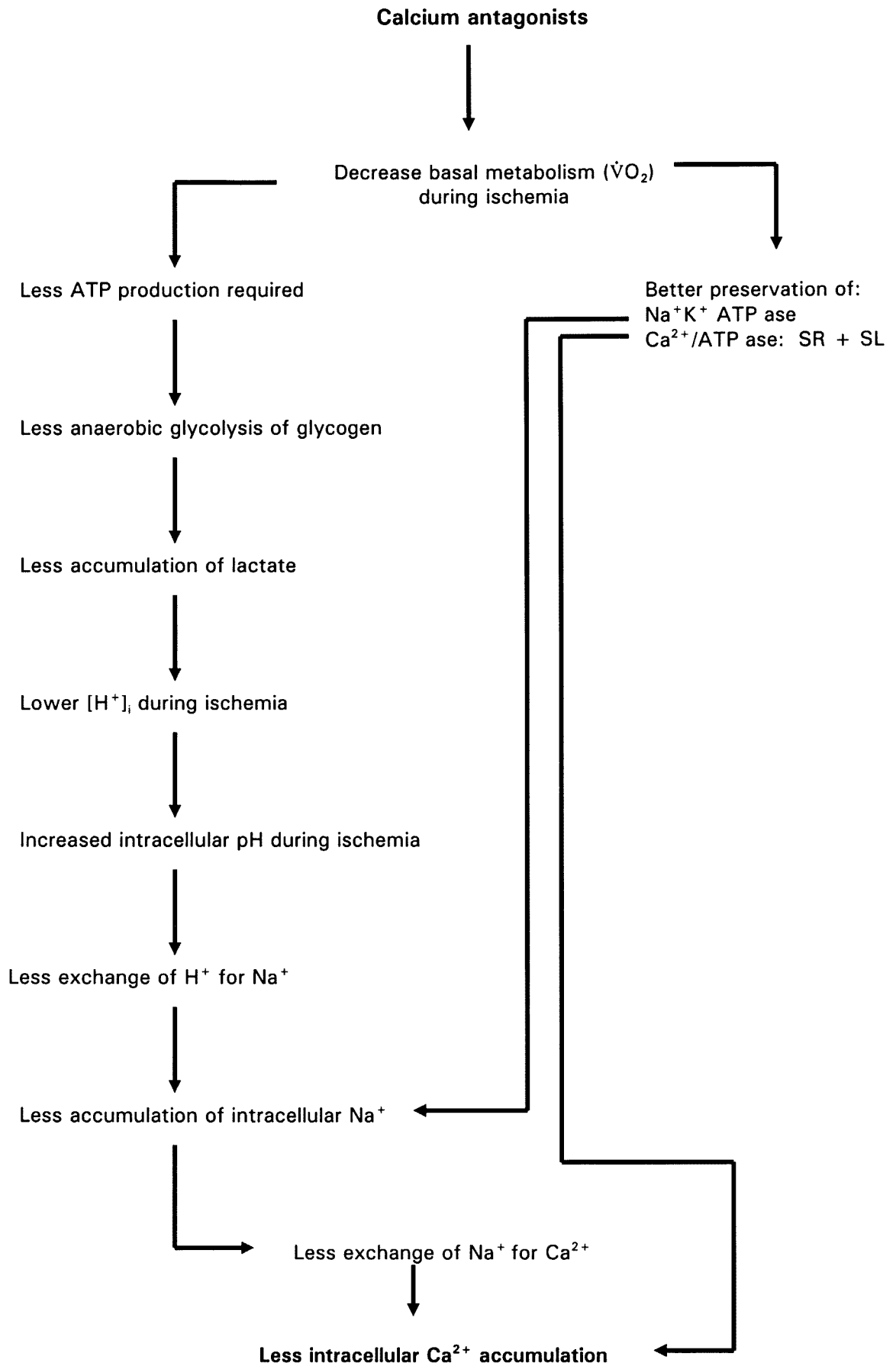
SR – sarcoplasmic reticulum
SL – sarcolemma

These changes during ischemia occur within the first 5 – 10 minutes of ischemia due to ionic changes, therefore their contribution to injury only occurs early during occlusion (Imai 1991, Ver Konck 1993, Silverman 1994).

Smart (1997) reported that reversal of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger contributed to stunning early during occlusion rather than after reperfusion. In Smart's study, amiloride (inhibitor of $\text{Na}^+/\text{Ca}^{2+}$ exchanger) was only protective if started before and administered throughout occlusion. The prevention of stunning correlated with inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange during the first 5 – 7 minutes of ischemia. Smart showed that after 7 minutes of ischemia, and during reperfusion, inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange had no beneficial effect on stunning.

Smart's study supports findings during myocardial hypoxia. During hypoxia as ATP is depleted, intracellular Na^+ rises, leading to a reversal of $\text{Na}^+/\text{Ca}^{2+}$ exchange and resulting in intracellular calcium overload. Studies confirmed that inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange reversal during hypoxia, rather than after reoxygenation, prevents injury (Zeigelstein 1992, Haigney 1992).

The effects of calcium antagonists during ischemia can be summarized in a schematic fashion:



Intracellular calcium increases further upon **reperfusion** due to reactivation of L-type calcium channels and consequent calcium release via the calcium overloaded sarcoplasmic reticulum, before re-uptake has been restored (Tani 1990, Sperelakis 1988, Opie 1992). The sarcoplasmic reticulum accounts for more than 90% of the calcium transient (Wier 1990, Barry 1993). The sarcoplasmic reticulum calcium content and release is regulated by the sarcolemmal L-type calcium channels (Barry 1993, Beukelmann 1988, Sperelakis 1988).

Calcium channel antagonists often have no effect on post-ischemic dysfunction when infused at or after reperfusion. However, important in this regard are: (Smart 1997)

- The fact that inhibitors are not effective for several minutes after administration, so infusion at reperfusion may not be effective during the critical first few minutes of reperfusion.
- The fact that an **adequate concentration** of drug needs to be present during the critical periods: Early during ischemia or at the onset of reperfusion.

Smart (1997) argued that an intracoronary nifedipine infusion starting 2 minutes before until 5 minutes after reperfusion (after 15 minutes of ischemia), would attenuate the initial burst of calcium influx due to reactivation of the L-type calcium channel on reperfusion. Consequently the sarcoplasmic reticulum

calcium release would be attenuated. In Smart's study stunning and ventricular fibrillation were prevented by a large dose intracoronary nifedipine infusion started 2 minutes before reperfusion until 5 minutes after reperfusion. Smart (1997) concluded that reactivation of myocardial L-type calcium channels at reperfusion contributes to both myocardial stunning and ventricular fibrillation on reperfusion, whereas increased intracellular calcium accumulation during ischemia due to reversal of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger contributes only to stunning.

4.7 The effect of verapamil on reperfusion arrhythmias.

The second important component of the reperfusion injury is arrhythmia. This is a common occurrence during the first few minutes of reperfusion, and is almost always ventricular in origin (Becker and Ambrosio 1987).

Calcium antagonists have been shown to reduce reperfusion arrhythmias. Smart (1997) reported that an intracoronary infusion of nifedipine from 2 minutes before to 5 minutes after reperfusion resulted in a lower incidence of ventricular fibrillation on reperfusion in the canine model subjected to 15 minutes of coronary artery occlusion followed by three hours of reperfusion. Verapamil also exerts a protective effect against ventricular fibrillation (Sherman 1981, Temeszy-Armos 1985, Brooks 1980, Opie 1988). This protection may be due to an increase of ventricular fibrillation threshold (Brooks 1980, Opie 1983) and a reduction in conduction delay (Elharrar 1977, Nakaya 1981).

In the present study the administration of 2mg of verapamil, either from the onset of ischemia, or from 3 minutes before reperfusion, abolished ventricular fibrillation compared to saline infusion (Table 3.7). None of the 13 animals that received 2mg of verapamil, developed ventricular fibrillation on reperfusion, whereas the incidence of ventricular fibrillation in the two saline groups was 40% (6/15). The lignocaine dosage required per animal to control ventricular dysrhythmias was 14.62mg in the verapamil groups and 54mg in the saline groups.

Smart (1997) in his study reported an incidence of ventricular fibrillation of 50% in control animals upon reperfusion and 38% if the intracoronary nifedipine was administered after reperfusion. However, as in the present study, Smart reported that pre-treatment with nifedipine (40 minutes before occlusion to 30 minutes after reperfusion) or administration of intracoronary nifedipine from 2 minutes before until 5 minutes after reperfusion, prevented reperfusion ventricular fibrillation. Bay K 8644 is a calcium channel agonist with an effect on calcium channels opposite to that of nifedipine. If Bay K 8644 was added to the intracoronary nifedipine infusion in the group where nifedipine was administered from 2 minutes before until 5 minutes after reperfusion, the incidence of ventricular fibrillation was 63%. If Bay K 8644 was substituted for nifedipine, the incidence of ventricular fibrillation on reperfusion was 75%. This finding strongly suggests that the initial burst of calcium influx via the L-type calcium channels at the onset of reperfusion mediates reperfusion ventricular fibrillation.

Brooks (1995) demonstrated that the initiation of ventricular tachycardia/fibrillation upon reperfusion was immediately preceded by large increases in the amplitude of the calcium transient. Abrupt increases in peak cytosolic intracellular calcium concentration are clearly associated with the initiation of reperfusion induced arrhythmias. Several other studies of beat to beat measurements of calcium (Kihara 1989, 1991, Thandroyen 1991, Smith 1988) have shown an association between an increase in intracellular calcium concentration and the induction of ventricular fibrillation. Intracellular calcium overload on reperfusion induces oscillatory calcium release from the sarcoplasmic reticulum (Opie 1988). Ryanodine (which inhibits sarcoplasmic reticulum calcium release), decreases the incidence of ventricular fibrillation in rat hearts on reperfusion, following regional ischemia (Thandroyen 1988).

Evidence further supporting the concept that changes in intracellular calcium concentration are important in the induction of reperfusion ventricular fibrillation are observations by Hirayama (1993) that verapamil significantly attenuates the magnitude of electrical alternans in vivo, and by Kimura (1986) that verapamil reduces the incidence of rapid ventricular activity on reperfusion.

Verapamil does not alter vulnerability to ventricular fibrillation in the non-ischemic/reperfused heart (Brooks 1980, Huang 1977, Fondacro 1978). This suggests that the protection afforded by verapamil is attributable to

pathophysiological changes that occurred during ischemia and especially at the time of reperfusion.

The importance of the role of calcium and the L-type calcium channels in the development of reperfusion arrhythmias was demonstrated in a study of the electrophysiologic consequences of both regional and global ischemia and reperfusion, by Kimura (1986) in the isolated cat myocardium. They reported the following: (Fig. 4.13).

During ischemia (30 minutes duration):

- The cell depolarizes: Resting membrane potential decreased by 21.6%.
- The action potential amplitude decreased by 29%.
- The action potential duration decreased by 54%.
- Ectopic activity occurred after 5 to 10 minutes (more frequent in regional than global ischemia), but rapid ventricular activity was **rare during** ischemia (17%).

On reperfusion:

- Rapid ventricular activity occurred in 83% of cases (global and regional ischemia), starting from 5-40 seconds after reperfusion and stopping spontaneously after ± 2 minutes.
- All cellular electrophysiologic changes seen during ischemia returned to baseline within 5 minutes of reperfusion.

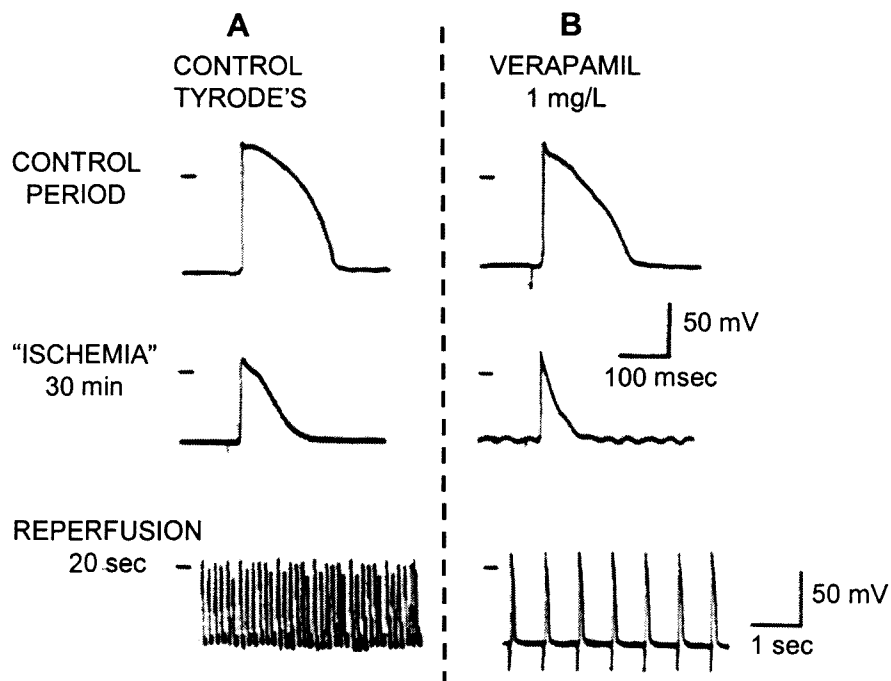


Figure 4.13

Effects of verapamil on transmembrane action potential changes during ischemia and rapid ventricular activity during reperfusion.

A: Transmembrane action potential changes during ischemia and the first 20 seconds of reperfusion.

B: Transmembrane action potential changes during the second ischemia-reperfusion Period in the **presence of verapamil**.

Verapamil did not have an effect on the action potential during ischemia but prevented the reperfusion induced rapid ventricular activity.

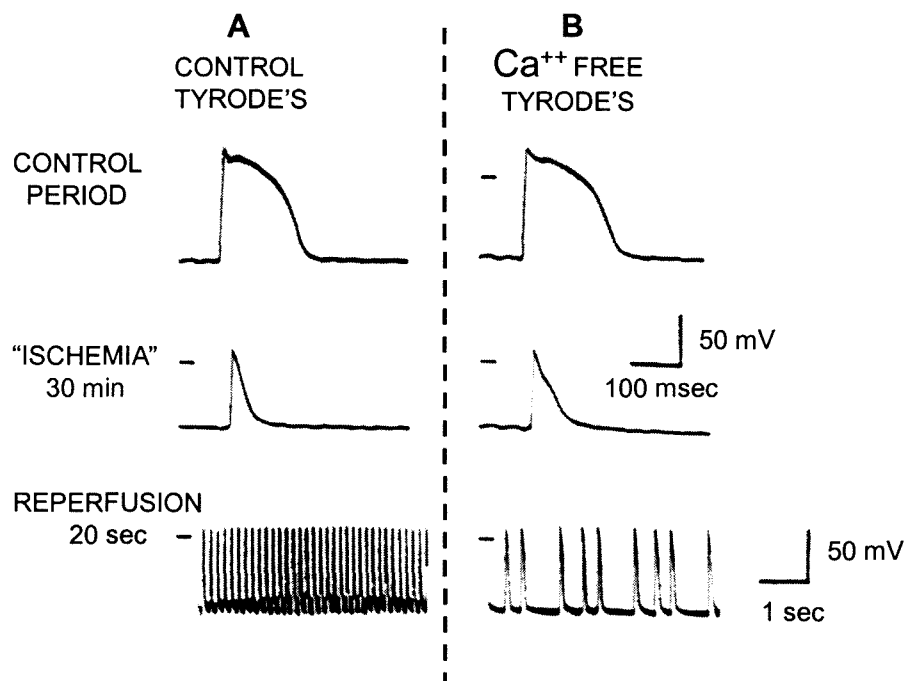


Figure 4.14

Effect of calcium free Tyrodé s solution on transmembrane action potential changes during ischemia and reperfusion ventricular activity.

A: Transmembrane action potential changes during the first ischemic period and rapid ventricular activity observed during the first 30 seconds of reperfusion.

B: Changes in transmembrane action potential and rapid ventricular activity during reperfusion **in the presence of the calcium free solution.**

Calcium free solution had similar effects as verapamil i.e. no effect during ischemia but successfully preventing the rapid ventricular activity.

Kimura concluded that verapamil (1mg/l) did **not** affect the electrophysiologic changes **during ischemia**, but **did** prevent reperfusion induced rapid ventricular activity (a lower concentration of verapamil, 0.1mg/l was not as effective). Perfusion with calcium free solution just before ischemia and during reperfusion also did **not** affect the electrophysiologic changes **during ischemia**, but **did** suppress reperfusion induced rapid ventricular activity (Fig. 4.14).

The author concludes that the ventricular fibrillation seen on reperfusion is due to rapid calcium cycling by the SR, which is triggered by calcium entry through the L type calcium channels at the time of reperfusion.

This study demonstrates that if the L type calcium channels are blocked by verapamil at the time of reperfusion, ventricular fibrillation does not occur. (Table. 3.7)

Conclusion and proposed clinical application.

Verapamil is effective in attenuating the reperfusion injury by means of separate effects, both during ischemia and at the time of reperfusion.

To gain maximum benefit from verapamil administration it is important that the drug be present in the ischemic myocardium, in adequate concentration, both

during early ischemia and before reperfusion, without adversely affecting systemic hemodynamics.

During cardiac surgery there is direct access to the coronary vasculature to exactly those areas at risk of ischemia (and hence reperfusion). This poses an ideal scenario for the clinical application of the findings of this study. In addition, because of the small amounts of drug delivered directly into the myocardium, it is speculated that the effects on the systemic hemodynamics would be negligible. In any event, if it should affect the systemic vasculature tone, it would be a simple matter to correct this with an alpha adrenergic agonist without affecting the loading conditions of the heart which, at that time, is excluded from the circulation.

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